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ROLE OF GENES AND THEIR RELATIONSHIP TO EXTRA-CHROMOSOMAL FACTORS IN THE DEVELOPMENT OF MAMMARY GLAND TUMOURS IN MICE.

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THE purpose of this paper is to give a discussion of the role of genes in the physiology leading to the development of mammary gland tumours in mice, and to emphasize the relationship of the genes to the extrachromosomal factors in determining the probability that the individual will develop a mammary tumour.

Development of the inbred lines with their characteristic tumour incidences together with hybridization studies have provided proof that genetic factors are influential in determining these incidences. Such incidences are expressions of the probabilities that females of the respective lines will have a mammary tumour. This probability may be extremely low in certain cases, such as in females of strain C_{57} black in which the incidence is less than 1 per cent, medium in other cases and extremely high in such mice as the females of strain C_3H, with an incidence of approximately 95 per cent.

It has been shown that aside from the genetic constitution, hormones and the milk agent are influential in controlling the probability that the tumour will appear. These cannot be considered as unrelated factors, but are normally interrelated in the physiology of the individual preceding the formation of the tumour.

In regard to the genetic influence, a mouse inherits a certain complex of genes whose actions manifest through certain physiologic paths affect the probability that a tumour will appear (Heston, 1946). One of these paths is through the physiology of hormone production, a second is through the physiology of the propagation and transmission of the milk agent, and a third possible path is through the physiology within the mammary tissue cell governing its reaction to the hormonal stimulation or the milk agent. Other paths may exist, and it is probable that these three are interwoven.

Proof of the effect of genic action in relation to the hormonal stimulation resulted from crosses between strain A in which the incidence in the virgin females is low although that of the breeders is high, and strain C_3H, in which the incidence in the virgin females as well as the breeders is high. Results of such crosses were reported by Bittner, Huseby, Visscher, Ball and Smith (1944), Heston and Andervont (1944), and Bittner and Huseby (1946). The fact that a high

incidence occurred among the virgin F_1 females resulting from mating strain A females to C_3H males as well as in the reciprocal F_1 virgins, together with evidence of segregation in the F_2 generation, indicated that the difference between the strains was primarily a genetic difference.

Such a difference was to be regarded as either a difference in hormone metabolism, or a difference in the reaction of the mammary gland cell to hormone stimulation. Such evidence as the fact that added oestrogen stimulation yielded tumours in strain A virgins, and the relationship of number of litters to tumour incidence in strain A reported by Jones (1940), strongly indicated a difference in hormone stimulation. Studies of the oestrus cycles of virgin females of strains A and C_3H and their reciprocal F_1 hybrids reported by Deringer, Heston and Andervont (1945), however, failed to show any difference in the cycles, except that in the low-tumour group of virgin strain A females the onset of oestrus was later than in the high-tumour groups. These observations, therefore, suggested that at least a part of the genetic difference could be manifested through the physiology within the mammary gland cell.

In an attempt to determine the physiological mechanisms through which the genetic differences are manifest, Huseby and Bittner (1947) also have carried out a number of studies, including ovarian transplantation to F_1 hybrid mice, ovariectomy, vaginal smearing, and histologic examination of the genital tissues. These studies have indicated that more than one endocrine organ is affected.

We have become more interested in the second path of gene action—that concerned with the propagation and transmission of the milk agent. It has been observed by a number of investigators that certain strains will propagate the agent, whereas others will not. All the high-mammary tumour strains normally transmit the agent from generation to generation, and even low-tumour strains may have the same innate capacity as Andervont (1945) has reported for strain C. The introduction of the agent transforms strain C into a high tumour strain. In Andervont's experience, however, strain C_{57} black does not have this capacity. While C_{57} black females that had nursed upon strain C_3H females could transmit the agent to susceptible young, their later generation C_{57} black offspring could not. On the other hand, Bittner (1948) has found tumours in his descendants of strain C_{57} black females into which the agent was introduced, and Fekete and Little (1942) have reported mammary tumours in descendants of C_{57} black females that had developed in strain dba uteri from transferred ova. Thus the situation in regard to the capacities of the various strains to propagate the agent is not clear.

By crossing high-tumour strain C_3H females with low tumour strain C_{57} black males, and comparing the two types of backcross females produced by backcrossing the F_1 females to both the C_3H and C_{57} black males, definite evidence of genetic differences in the ability to propagate and transmit the agent was revealed (Heston, Deringer and Andervont, 1945). A significantly higher tumour incidence was observed in the high-tumour strain backcross females (C_3HBC) than in the low-tumour strain backcross (BBC), although both groups had been born from comparable F_1 mothers and had received comparable milk agent. Furthermore, the C_3HBC females could transmit the agent better than the BBC females, as shown by tumour development in uniform test females fostered upon both types of the backcross females. Since other factors were controlled, this difference in the ability to propagate and transmit the agent had to be attributed

to the genetic differences of the backcross females produced by the differences in their fathers.

Individual variation in the ability to transmit the agent was particularly apparent in the BBC females. There was not all or none expression, however, but the variation was expressed in degree suggesting the effect of several genes.

An important field of gene-extrachromosomal agent relationship has thus been opened, and is being extensively investigated at the present time. The possibility of an intracellular origin of the agent also enters into consideration. In some respects the picture is comparable to the gene-kappa relationship studied by Sonneborn (1945) and co-workers in paramecium. The picture is not as simple, however, in that there is only a single gene associated with kappa.

It is emphasized again that these differences are expressed as differences in the probability that the tumour will occur. Changes in the genetic constitution may result in a change in this probability. Variations in the hormone stimulation result in variation in this probability. We recently have been obtaining results indicating that the effects of removing the milk agent likewise must be considered as merely lowering the probability that a tumour will arise. Our attention has been directed toward tumours that arise in females in which the agent cannot be demonstrated (Heston, Deringer and Levillain, unpublished data).

In order to obtain a line of strain C_3H free of the milk agent a litter of 3 females and 3 males were removed from their C_3H mother by Cesarean section and fostered upon C_{57} black female. These females were later mated to their male littermates, and subsequently the line has been maintained by brother $\times$ sister matings, the young being nursed upon their own mothers. In this line, designated as C_3Hb, mammary tumours are arising. In 194 of the females of this line 14 months of age and older and extending through the first 5 generations to date, 47, or 24 per cent, have developed mammary tumours at an average age of 18·9 months; seventy-eight have died without mammary tumours at an average age of 16 months, and 69 are still living at an average age of 18 months.

Study of the histologic section of 37 of these tumours has not revealed much dissimilarity between these and the usual C_3H mammary tumours. Nine, however, have shown areas of squamous metaplasia with pearl formation similar to that described by Kirschbaum, Williams and Bittner (1946) in mammary tumours induced by methylcholanthrene in the absence of the milk agent Such areas also occasionally occur in normal strain C_3H females of older age, suggesting that the occurrence of squamous areas may be characteristic of older age rather than specifically owing to the possible absence of the milk agent. Three of the tumours arising in these C_3Hb females have been transplanted into other C_3Hb females, and in each case the transplant has grown.

We have no evidence, however, of a milk agent in the etiology of these tumours. Distribution of the tumour-bearing females in the pedigree chart does not show segregation of tumour families, as would be expected if the agent were present. Furthermore, we have found no evidence of the agent in cell-free extracts of these tumours. These extracts varied from 2 to 5 per cent, and were prepared in 0·005 M phosphate buffer pH 7·0 with a Waring blendor; cleared in a multispeed centrifuge at 18,000 g. for 3 minutes; and filtered through a Berkfeld H or an 8 lb. Mandler filter. To test for the agent 0·1 c.c. of the filtrate has been injected into C_3Hb females at from 1 to 5 days of age. Many of these test females are not beyond the expected tumour age, but those for three of the tumours are 14

months of age and older, and none has yet developed a tumour. Extracts prepared with the same technique from tumours from C_3H females produce tumours in C_3Hb test females in approximately 8 to 10 months.

This is not presented as absolute proof that the agent is not present. If it is, however, it is not comparable to that in C_3H females, nor is there any evidence of its being built up comparable to that of C_3H. If the agent is not present we have proof that the accumulation of genetic factors and of non-genetic factors other than the milk agent can cause the tumour threshold to be surpassed. In this light the milk agent would be comparable to the carcinogens in the induction of lung tumours, where the presence of the carcinogen appears to merely increase the probability that the tumour will appear and at an earlier age (Heston, 1942*a*,*b*).

While all of these factors have been shown to alter the probability that the normal mammary tissue cell may be transformed into a malignant cell, these investigations have not answered the question as to what constitutes this change to malignancy. To a geneticist the probability aspect of the picture and the manner in which the probability can be altered at once suggests a mutation. Recent advances in genetics suggest that this may not necessarily be a gene mutation, but may be a mutation of cytoplasmic factors. Since the change is normally irreversible, it must be a change in the fundamental physiology of the cell. It is gratifying that the way has been opened for obtaining experimental evidence bearing on this point, some of which will be reported later in this symposium.

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GENETICALLY DETERMINED DIFFERENCES IN HORMONE PRODUCTION A POSSIBLE FACTOR INFLUENCING THE SUSCEPTIBILITY TO MAMMARY CANCER IN MICE.

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It was shown by Little *et al.* (1933) and Korteweg (1933) that the susceptibility to cancer of the mammary glands of mice is partly determined by a non-genetic extrachromosomal influence. That this influence is transferred from mother to young by means of the milk was later proved by Bittner (1936). Korteweg (1936*a*, *b*) predicted that chromosomal factors must also be active. This prediction was based on the fact that differences in susceptibility to cancer exist between dba females and the F1 hybrids of the dba × C57 black cross. The present paper concerns these chromosomal factors.

The fact that chromosomal factors exist is important, but the way they act, their mechanism, is of still greater interest. A number of investigators have considered the role of the follicular hormone, and checked the possibility of differences between the oestrus cycles of the high-cancer and low-cancer strains of mice. As no differences were found this form of experiment was discontinued.

Yet I was not convinced. I spayed females of different strains, and afterwards determined the sensitivity of the vaginal epithelium to oestrone by means of the vaginal smear method. I found that, to cause oestrus, it was necessary to inject three times as much oestrone into females of the high-cancer strain dba as in those of the low-cancer C57 black. As the course of natural oestrus in both these strains is the same, I concluded that normal dba females produce three times as much oestrone as normal C57 females. I suggested (Korteweg, 1935) that this overproduction of oestrone in dba females might enhance their susceptibility to cancer. Since these results were published in 1935 this line of research has been continued with the aid of my co-workers, van Gulik and Mühlbock, up to the present time, interrupted only by war circumstances.

Our low-cancer strain 020 also proved to be very sensitive to oestrone, as judged by the oestrus test in spayed females. The females of this strain therefore probably produce only relatively little of this hormone (van Gulik and Korteweg, 1940). In accord with this result, Shimkin and Andervont (1941) found their low-cancer strain C57 black much more sensitive than their high-cancer strain C3H. On purely anatomical grounds Fekete (1946) concluded that dba mice produce more oestrone than C57 females. I can confirm her findings. The ovaries of our high-cancer strain greatly surpass those of our low-cancer animals in volume. This is partly caused by the somewhat greater number of graafian follicles, partly by the much greater number of corpora lutea, and partly by the

existence of large follicular cysts in dba mice. According to Nathanson of Boston (personal communication) the ovaries of the high-cancer strain C3H closely resemble those of the dba strain.

Since in the reciprocal F1 hybrids between dba and C57 the sensitivity to oestrone was the same and about intermediate between the sensitivity in the parent strains, we concluded that these differences in sensitivity to oestrone had nothing to do with the milk factor (van Gulik and Korteweg, 1940). Shimkin and Andervont (1941), by means of foster nursing experiments, reached the same conclusion.

At the present time we know from the experiments on oestrus response to injected oestrone in spayed females that in mice of two high-cancer strains more oestrone is being produced than in those of two low-cancer strains, but from this we may not yet conclude that the production of a larger quantity of oestrone is a quality of mice of all high-cancer strains. This remains to be seen, and the present communication is therefore only of a preliminary character.

Here a digression is necessary. The general opinion seems to be that whereas in the human after puberty there are both oestrogenic and luteal ovarian phases, there is in unmated mice no luteal change (Pullinger, 1947). This surely is not true. Hooker (1945) recorded that the effect of progesterone can be demonstrated in the structure of the endometrial stroma just as well as the effect of oestrone. I have found that the epithelium of the uterus of the virginal mouse also shows a response to progesterone. As in the endometrium of the human female, in which on the 17th day of the cycle, shortly after the bursting of the follicle when the luteinizing process is beginning, a vacuole becomes visible in the basal part of the epithelial cells, so also this phenomenon occurs in the virginal mouse. It is therefore necessary to be aware always of the possibility that progesterone too may influence susceptibility to cancer (by its synergistic action with oestrone).

If the mammary glands also of high-cancer strain mice should be relatively insensitive to oestrone, as is the vaginal epithelium of high-cancer strain animals, then the excess of oestrone produced would probably be harmless to the mammary glands. If, on the contrary, the sensitivity of the mammary glands of high- and low-cancer strain animals should be the same, the excess of oestrone produced in high-cancer strain animals might be injurious to the mammary glands. It therefore became necessary to determine the sensitivity to oestrone of the mammary glands. As the result of injecting a total of 108 I.U. of oestrone into 2½-months-old spayed females of different strains, it seemed evident to van Gulik and myself that our high-cancer strain females dba reacted to a lesser degree to oestrone than our low-cancer strain animals when judged by the extent of development of the glands. In 2½-months-old castrated males injected with 13 I.U. of oestrone, we found more growth also in the low-cancer than in the high-cancer strains. Mühlbock then drew our attention to the fact that in our experiments we had injected large doses. If one wishes to determine the sensitivity of an organ it is preferable to determine the threshold dose. As the first visible sign of an effect of oestrone, Mühlbock (1948*a*) took the beginning of budding of the end of the milk ducts (swelling of the end bulbs). He injected non-castrated 5-months-old males of our three strains with different doses of oestrone. Again more oestrone was needed in the high-cancer than in the low-cancer strain animals.

All previous experiments seemed to prove that in high-cancer strain animals the mammary glands are relatively less sensitive to oestrone than are those of low-cancer strain mice (Korteweg, 1947). Nevertheless, when determining the threshold dose of oestrone in spayed 6 weeks-old females Mühlbock (1948*b*) found by this test that the mammary glands of high-cancer strain females are just as sensitive as those of low-cancer strain mice. Males of the same strains have also been examined by Mühlbock (1948*a*). Two facts appeared. Firstly in the males of the three strains C57 black, dba and 020, the sensitivity of the mammary gland is about the same according to the threshold test. Secondly, the minimal dose causing budding (swelling of end bulbs) in castrated males is approximately five times less than in non-castrated males. Obviously in non-castrated males the testosterone largely counteracts the oestrone. This supposition proved to be right, as in castrated males which were injected both with oestrone and testosterone, the presence of the latter suppressed the action of the former. In non-castrated males three times as much oestrone is needed to cause budding (swelling of end bulbs) in the glands of high-cancer strain dba mice than in those of low-cancer strain mice ; in castrated males the sensitivity is the same. The only possible conclusion to be drawn from these facts seems to be that in the high-cancer strain males which were examined, the quantity of testosterone produced exceeds that of the low-cancer strain males by three times. This relatively high production of oestrone in females and of testosterone in males of our high-cancer strain suggests that at the bottom of this phenomenon there exists a relatively high production of gonadotrophic hormone in this strain. This gives a hint that from now on our attention should be fixed especially on the hypophysis.

SUMMARY.

It was found by the oestrus test in spayed females that our high-cancer strain animals produce relatively large amounts of oestrone in comparison with low-cancer strain mice. This excess of oestrone acts on a mammary gland which is just as sensitive to the action of this hormone as is the gland of low-cancer animals when sensitivity is judged by the threshold response at 6 weeks old. That means that the mammary glands of our high-cancer strain females are exposed to abnormal stimulation by oestrone. It therefore seems probable that at least part of the genetically determined disposition to mammary cancer in certain strains of mice is caused by an overproduction of this hormone.

If in the A strain, with its great difference in cancer incidence between virgins and breeders, the production of oestrone should prove to be low, then the differences in production demonstrated by us might be identified with the so-called " inherited hormonal influence " referred to in the literature. If, on the contrary, the production of oestrone in the A strain should prove to be high, and if the same should be the case in the other high-cancer strains, then these differences of oestrone production will have to be identified with another, not yet determined genetic factor.

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MAMMARY TUMOUR INDUCING FACTOR AND GENETIC CONSTITUTION.

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SINCE Bittner (1940) first postulated that at least three factors, namely, the milk factor, the hormonal factor, and the genetic factor or genetic constitution, play a part in the origin of breast cancer in inbred strains of mice, in a number of laboratories a great amount of work has been carried out on each of the factors. The interaction of all three factors in the development of breast cancer in mice has been clearly shown (Bittner, 1945) and the influence of other factors like abnormal environmental and metabolic conditions has been described (Morris, 1945).

Experiments showing the interaction of mammary tumour inducing or milk factor and genetic constitution in the development of breast tumours in certain high- and low-breast cancer strains of mice will be described in the present paper, and the importance of the genetic factors shown.

Experiment No. 1.

Susceptibility to the milk factor begins to decrease gradually after birth, and susceptible mice after they reach maturity are quite resistant to the action of the milk factor (Bittner, 1942*a*; Andervont, Shimkin and Bryan, 1942.)

Following these observations, an experiment was carried out to find out whether it would be possible by administering sufficient quantities of milk factor in tumour tissue from RIII and Strong A high-breast-cancer strains to induce breast cancer in susceptible C57 × Strong A hybrid females which had not obtained the factor while suckling and were 16 weeks old. Litter mates of these mice, 4–6 weeks old, were used as controls. The two age-groups of C57 × Strong A hybrid females, each were subdivided into two groups according to the number of subcutaneous injections of distilled water suspensions of dried RIII or Strong A breast tumour tissue. Mice in each separate group were given equal quantities

of tumour tissue from RIII and Strong A strains. Each mouse was given at each injection 0·5 c.c. of the suspension containing an amount of tissue corresponding to 0·125 g. of fresh breast tumour tissue (Dmochowski, 1945*a*).

The results are shown in Table I. As can be seen, the resistance of adult susceptible mice can be overcome by a large dose of the milk factor, and a high

TABLE I.—*Induction of Breast Cancer in Susceptible C57 × Strong A Hybrid Females of Different Ages by Administration of Milk Factor in Dried Strong A and RIII Breast Tumour Tissue.*

Number of mice.	Age of mice when first injected (weeks).	Number of injections.	Origin of milk factor (dried breast tumour tissue).	Group of mice.	Tumour incidence.	Percentage.	Average tumour age (months).
8	4–6	4	Strong A	A	5 out of 8	62·5 (SE = 17·12)	17
15	4–6	4	RIII	B	14 out of 15	93·3 (SE = 6·48)	15
10	16	4	Strong A	..	1 out of 10	10	28
12	16	4	RIII	..	0	0	..
11	4–6	12	Strong A	E	8 out of 11	72·7 (SE = 13·45)	17
10	4–6	12	RIII	F	8 out of 10	80·0 (SE = 12·65)	15
34	16	12	Strong A	C	20 out of 34	58·8 (SE = 7·99)	18
20	16	12	RIII	D	17 out of 20	85·0 (SE = 8·44)	15·5

Group A and B: Standard Error of difference = 18·19, difference = 30·8. The ratio of the difference between the two proportions to the Standard Error of that difference is 1·7; therefore it is not quite certain whether the difference has not arisen by chance.

Group E and F: SE of the difference = 18·46, difference = 7·3. This result is obviously not significant.

Group C and D: SE of the difference = 11·62, difference = 26·2. Therefore, the ratio of the difference to the SE of the difference is 2·25, which means that the observed difference between the tumour incidence induced by the milk factor of these two strains is significant.

This table is a summary of the present and previously published results (Dmochowski, 1945*a*).

incidence of breast cancer can be induced in these mice. It is not necessary, therefore, for the milk factor to take part in the development of mammary glands of susceptible mice to induce a high incidence of tumours, and an increased liability to breast cancer can be induced in adult susceptible mice by the factor, providing the mammary glands are subjected to the influence of large amounts of the factor.

These results confirmed previous findings (Dmochowski, 1944*a*) that mammary tumours can be induced in susceptible mice which are of an entirely different genetic constitution from the strain from which the milk factor originated.

The influence of genetic constitution may be seen in the differences in the milk factor present in the breast tumour tissue of RIII and Strong A high-breast-cancer strains (Table I). Equal amounts of the milk factor from the two different high-breast-cancer strains induced a different breast tumour incidence in the same susceptible mice. Similar results were also obtained in other susceptible mice given the milk factor of the same two high-breast-cancer strains (Dmochowski, 1945*b*). Thus, administration of the milk factor from two different high-breast-cancer strains to the same susceptible hybrid mice, kept under controlled conditions, has shown differences in either quantity and/or quality of the factor present in these two high-breast-cancer strains.

Bittner and Huseby (1946) reported similar findings by a different experimental procedure, namely, by reciprocal crossings of two high-breast-cancer strains (C3H and Strong A), and by recording the tumour incidence and tumour age in virgin hybrids obtained from these strains. They described the recorded differences in the tumour incidence and in the tumour age in these hybrids as a result of different concentration and/or activity of the milk factor present in these two high-breast-cancer strains. Heston and Andervont (1944) in a similarly conducted experiment on sublines of the same strains found no conclusive evidence of a difference in the milk factor of these strains. Andervont and McEleney (1941) pointed out, however, that his high-breast-cancer strain (C3H) must be regarded as a separate line, and the results obtained with it cannot be controlled by data procured from other lines of this strain. Warner, Reinhard and Goltz (1945) in experiments on virgin hybrids of two other high-breast-cancer strains (M and A) obtained similar results, and concluded that the milk factor was more concentrated in one of these strains than in the other. Bittner and Huseby (1946) found, however, the same incidence of breast cancer in breeding hybrids of the two strains tested. They interpreted this as a possible result of an increased hormonal stimulation which could make the breast tissue more sensitive or produce an increased amount of the milk factor. Breeding hybrid mice did show a difference in the tumour incidence providing the maternal high-breast-cancer strains differed considerably in their tumour incidence (Bittner, 1936).

Murray (1941) pointed out that the differences in mammary tumour incidence of various inbred strains may be due to the variability of the milk factor. Experiments carried out by Murray and Little (1939) suggested that the differences in the milk factor may be due to the genetic constitution of the strains which were examined. Khanolkar and Ranadive (1947), in their studies of microscopical structure of breast tissue of three inbred strains, recently found that the milk factor of these strains appears to vary in quality or quantity, confirming the present results. But they rightly pointed out that the ultimate result of the changes in the structure of breast tissue acted upon by the milk factor of another strain is determined also by the genetic constitution and responsiveness of the breast tissue to the particular milk factor.

Heston, Deringer and Andervont (1945), by employing susceptible hybrid mice obtained from high-breast-cancer strain mothers and low-breast-cancer strain fathers, and by backcrossing them to high- and low-breast-cancer strain males, showed that differences in the milk factor were produced by differences in the genetic constitution of the mice from which the milk factor originated. They also advanced the view that the location of the genic action in controlling the susceptibility of mice to breast cancer occurs in mammary tissue cells.

Thus the genetic constitution of mice has an influence on the production of the milk factor, and on the susceptibility or resistance of mice to this factor.

Experiment No. 2.

The influence of genetic constitution on the degree of susceptibility or resistance of mice to the milk factor may be seen from the following experiment.

Following the observation that a high incidence of breast cancer can be induced in adult susceptible mice with large doses of the milk factor, an experi-

ment was carried out to ascertain how adult low-breast-cancer strain mice will react to similar quantities of the factor.

C57 black low-breast-cancer strain mice which were litter mates were divided into two groups : first, comprising 48 mice, 4–6 weeks old, and second, comprising 30 mice, 16–20 weeks old, which had given birth to one or two litters. Each mouse of both groups received 14 subcutaneous injections, corresponding to 1·4 g. of fresh RIII breast tumour tissue. Mice in both groups were forcibly bred. The injections were of dried RIII breast tumour tissue resuspended in distilled water.

The results are shown in Table II. No tumours developed in any mice of the two groups of C57 black low-cancer strain mice. While large doses of the milk

TABLE II.—*Administration of Milk Factor in Dried RIII Breast Tumour Tissue to C57 Black Low-Cancer Strain Females of Various Ages.*

Number of of mice.	Age of mice in weeks when first injected.	Number of injections.	Incidence of tumours (%).	Average life-span in months.
48	4–6	14	0	18·1
30	16–20	14	0	17·7

factor can break down the resistance of adult susceptible mice, the milk factor in amounts far exceeding those required to induce breast cancer in young susceptible mice has no influence on 4 weeks to 4 months old low-breast-cancer strain mice.

The genetic factors, which determine the susceptibility or resistance of mammary tissue cells to the milk factor, may be responsible for the breast tumour incidence in mature susceptible mice under the influence of great quantities of the milk factor, and also for the failure of similar large quantities of the milk factor to induce breast cancer in mature C57 black low-breast-cancer strain females.

It must be stressed, however, that differences in the genetic factors acting through the hormonal mechanism of these strains, and also differences in the hormonal factors of the strains examined, may have to be taken into consideration in interpreting the present results. While there seems to be little doubt that the genetic constitution of mice influences the reaction of breast tissue to the milk factor, the possibility of a milk factor deterrent present in C57 mice with the resulting ability to counteract and destroy the milk factor must also be considered.

Sublines of the C57 black low-breast-cancer strain vary in the degree of susceptibility to the action of the milk factor. This explains the results of Bagg (1939), Little and Pearsons (1940), and Andervont (1943) of foster-nursing C57 black mice by high-breast-cancer strain females and their forced-breeding with the resulting breast tumour incidence varying from 14 per cent to 63 per cent. C57 black mice employed in the present experiments when foster nursed by RIII high-breast-cancer strain females give an incidence of breast cancer of 11 per cent (Dmochowski, unpublished data). It seems that the low susceptibility of this subline of C57 black mice may be, at least partially, responsible for the results of the present experiment when even the forced breeding, combined with large doses of the milk factor, failed to induce breast tumours in mature C57 black low-cancer-strain females of different ages.

Experiment No. 3.

Heston, Deringer and Andervont (1945) pointed out that besides the genetic factors responsible for the susceptibility to the milk factor, there may be still another set of genetic factors responsible for the propagation and transmission of the milk factor.

The influence of the genetic constitution on the propagation and transmission of the milk factor may be seen in the following experiment.

S low-breast-cancer strain females, which have a normal incidence of breast cancer of less than 1 per cent, and give a 29·6 per cent incidence of breast cancer when nursed by RIII females (Dmochowski and Gye, 1943), were employed (Fig. 1). The S strain females which were litter-mates were divided as equally

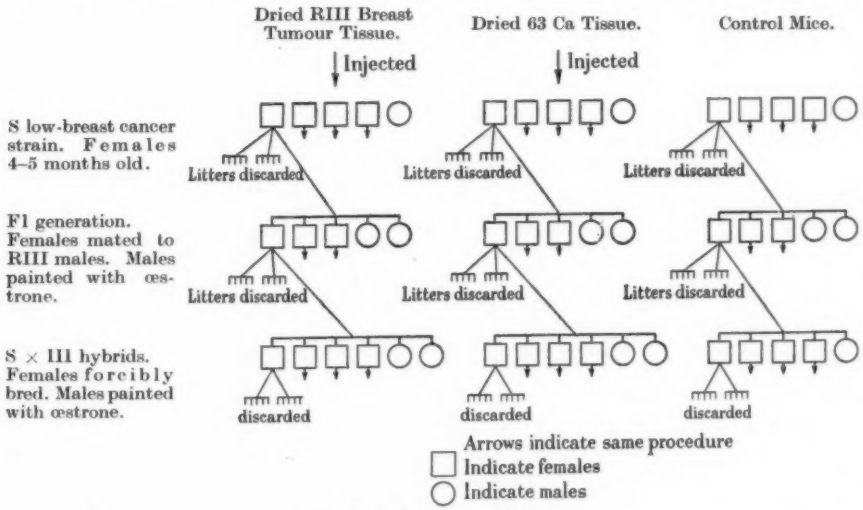

Fig. 1.—Experiment No. 3.

as possible into three groups. Mice of the first group, when 4–5 months old, received one subcutaneous injection of a distilled water suspension of dried RIII breast tumour tissue corresponding to 0·125 g. of fresh breast tumour tissue; mice of the second group, when of the same age, received one subcutaneous injection of a distilled water suspension of dried 63 Ca transplantable breast tumour tissue (from a 428th transplant of the tumour obtained from the Imperial Cancer Research Fund) corresponding to 0·125 g. of fresh breast tumour tissue; mice of the third group were kept as controls. In the F1 progeny of mice of all three groups the following procedure was employed: the first two litters were discarded, the following litters kept, and females mated to RIII high-cancer strain males, and their brothers painted with a 0·02 per cent solution of keto-hydroxy-oestrone in chloroform for 12 months. The hybrid mice obtained by mating F1 S strain females to RIII males were subjected to the following procedure which was the same in S × RIII hybrid mice of all three groups. S × RIII hybrid females were forcibly bred by removing the first two litters and then

allowed to breed normally, and S × RIII hybrid males were painted with 0·02 per cent solution of keto-hydroxy-oestrone for 12 months.

The results are shown in Table III. No tumours developed in any of the S low-breast-cancer strain females, whether injected with RIII or 63 Ca dried breast tumour tissue. These females lived to an average age of 17·8 months.

In the F1 generation of S strain females injected with 63 Ca tissue and of the control females, no tumours were observed in any of the females, or in any of the males painted with oestrone. In the F1 generation of S strain females injected with RIII dried tumour tissue, 7 out of 16 females developed breast cancer, and 5 out of 15 males, which were painted, also developed breast tumours.

In S × RIII hybrid females obtained from daughters of S strain females injected with dried RIII breast tumour tissue, 27 out of 32 females showed breast tumours, while S × RIII hybrid males of the same origin, painted with oestrone, gave tumours in 10 out of 16 mice. A breast tumour developed in 1 out of 32 S × RIII hybrid females derived from daughters of S strain females injected with dried 63 Ca breast tumour tissue, and no tumours in any of the 22 S × RIII males painted. In S × RIII hybrid females of the third group 3 out of 34 developed breast cancer, and in 2 out of 32 S × RIII hybrid males of the same group, which were painted, breast cancer was observed.

In this experiment the milk factor was transmitted to the progeny of adult S low-breast-cancer strain females which themselves did not develop breast cancer. This is of interest in view of the recent findings of Andervont (1945*b*), who observed that adult female mice of another low-breast-cancer strain (strain I) injected with the milk factor did not develop breast cancer, and did not transmit it to the progeny obtained by mating these females to high-breast-cancer strain males, while adult susceptible mice, after a single injection of the milk factor, did not develop breast cancer, but transmitted it to their progeny. Bittner (1942*b*, 1943, 1944) noted a difference in the tumour incidence of successive litters born to the same susceptible females, and mice belonging to the third and fourth litters showed a higher tumour incidence than those of the first litters. It would have been of interest to compare the incidence of breast cancer in the first and the third litters of S strain females injected with RIII tumour tissue to see if any difference in the tumour incidence could be noted. This could not be done owing to the limited space available and the large numbers of mice it would involve. Bittner (1942*a*) observed that low-breast-cancer strain females fostered by high-breast-cancer strain mothers could transmit the milk factor in their milk, although they themselves did not develop breast cancer. In the present experiment, it was noted that the occurrence of breast cancer in S × RIII mice of the first group was independent of the appearance of a tumour in their F1 S strain mothers. The increased tumour incidence in S × RIII hybrid mice of the first group is another instance of the interaction of the milk factor, genetic constitution, and the hormonal factor in the development of breast cancer in mice. The addition of susceptible strain chromatin from RIII strain males resulted in mice developing more tumours.

Thus the present experiment has shown that the milk factor can be transmitted through relatively resistant strain S mice and induce a high incidence of breast cancer in susceptible mice. Again, it would have been of interest to observe the incidence of breast cancer in S × RIII hybrid mice obtained from S strain females injected with RIII dried tumour tissue, and to compare it with that of

TABLE III.—*Administration of Dried RIII Spontaneous Breast Tumour and 63 Ca Transplantable Breast Tumour Tissue to S Low-Breast-Cancer Strain Females 4–5 months old.*

Origin of mice.	Dried RIII breast tumour tissue.					Dried 63 Ca transplantable tumour tissue.				Control mice.			
	Sex.	Number of mice.	Tumour incidence.	Percentage.	Average tumour age.	Number of mice.	Tumour incidence.	Percentage.	Average tumour age.	Number of mice.	Tumour incidence.	Percentage.	Average tumour age.
S strain, 4–5 months old	F.	8	0 out of 8	0	..	8	0 out of 8	0	..	8	0 out of 8	0	..
F1 S strain	F.	17	7 out of 16	(Group A) 43·8 (SE = 12·4)	16·14 (SD = 2·898)	14	0 out of 12	(Group E) 0	..	15	0 out of 13	(Group J) 0	..
	M. (painted)	18	5 out of 15	(Group C) 33·3 (SE = 12·167)	17·4 (SD = 1·369)	12	0 out of 10	(Group G) 0	..	16	0 out of 12	(Group L) 0	..
S × RIII hybrids from F1 S females mated to RIII males.	F. forcibly bred	38	27 out of 32	(Group B) 84·3 (SE = 6·4)	14·8 (SD = 5·094)	31	1 out of 31	(Group F) 3·2 (SE = 3·174)	15·8	42	3 out of 34	(Group K) 8·8 (SE = 4·86)	14·3
	M. painted	32	10 out of 16	(Group D) 62·5 (SE = 12·11)	15·6 (SD = 4·094)	27	0 out of 22	(Group H) 0	..	48	2 out of 32	(Group M) 6·25 (SE = 4·279)	13 8

Group A and B : SE of difference = 14·6, difference = 40·5. Group G and H : SE of difference = *nil*, difference = *nil*.
Group J and K : SE of difference = 4·86, difference = 8·5. Group C and D : SE of difference = 17·16, difference = 29·2.
Group E and F : SE of difference = 3·174, difference = 3·2. Group L and M : SE of difference = 4·279, difference = 6·25.

Dr. C. W. Dixon, of the Department of Preventive Medicine and Public Health, School of Medicine, University of Leeds gave the following observations :

"The incidence of tumours in groups A and B is of an order that would have occurred by chance alone only about 1 in 200 times. All other things being equal the figures therefore do support the view that the incidence in Group B is significantly greater than that in Group A.

"In comparing the incidence in groups C and D the difference in incidence is not significant for the sizes of samples used. In the comparison of Groups E and F, G and H, J and K, L and M there is, similarly, no significant difference in the incidence of tumours. In all these groups the numbers involved are very small, and clear-cut conclusions should not be atte pted from them.

"The average tumour age is shown in the columns with the standard deviation around the mean. Although at first sight it would appear that the S × RIII hybrids developed their tumours earlier, the considerable variation around the mean makes the straight arithmetic give an imperfect picture of the situation. In actual fact there is no significant difference in the tumour age between Groups A and B or C and D."

S × RIII hybrid mice obtained from mating F1 S strain females. This could not be done owing to the numbers of mice it would involve.

It seems that mice of some, at least, low-breast-cancer strains like mice of high-breast-cancer strains may differ in their susceptibility to the action of the milk factor and also in their ability to transmit and propagate it. The ability to transmit the factor may vary from 100 per cent like in the C3H low-cancer strain of Andervont (1945*b*), through C57 strain of Fekete and Little (1942), CBA strain of Miller and Pybus (1945), down to strain I of Andervont (1945*a*).

Thus the presence of two sets of genetic factors, one controlling the susceptibility of breast tissue cells to the milk factor either directly or through the hormonal mechanism, the other controlling the propagation of the milk factor, must also be considered in low-breast-cancer strain mice.

The low incidence of breast cancer observed in S × RIII hybrid females forcibly bred, and in S × RIII males painted with oestrone, whether derived from the progeny of S low-cancer strain control females or S strain females injected with dried 63 Ca tumour tissue, has been previously described (Dmochowski, 1944*b*). It would have been of interest to test these tumours for the presence of the milk factor. Similar observations on hybrid mice derived from low-breast-cancer strain females and high-breast-cancer strain males have been reported from several sources (Murray and Little, 1939; Bagg and Jacksen, 1937; Bittner, 1939; Strong, 1943; Andervont, 1945*c*; Foulds, 1947). There was no significant difference in the incidence of breast cancer in hybrid mice from the S strain control mice, and in hybrid mice from the S strain females injected with dried 63 Ca tissue. It seems, therefore, that an increased hormonal stimulation rather than any possible small amount of the milk factor in 63 Ca tumour tissue may play a part in the development of these tumours.

Experiment No. 4.

The absence of the milk factor in 63 Ca transplantable tumour is shown in Table IV. No tumours developed in C57 × RIII hybrid females injected with

TABLE IV.—*Induction of Breast Cancer in C57 × RIII Hybrid Females by Administration of Dried RIII Breast Tumour and Dried 63 Transplantable Breast Tumour Tissue.*

Number of mice.	Age of mice in weeks when first injected.	Number of injections.	Origin of breast tumour tissue.	Incidence of tumours (%).	Average tumour age in months.	Average age of mice without tumours in months.
16	4–6	16	63 Ca	0	..	16·9
36	4–6	1	RIII	82·1	9·6	17·8

large doses of 63 Ca dried tissue, corresponding to 1·6 g. of fresh tissue, while their litter-mate controls injected with dried RIII breast tumour tissue (amount corresponding to 0·125 g. of fresh tissue) developed a high incidence of breast cancer. The presence of even small amounts of milk factor should have been observed by the method employed in the present experiment. It is impossible to state, however, whether the milk factor was originally absent in the primary tumour or whether it was lost during the course of so many transplantations.

In conclusion, it can be stated that only an analysis of the action and interaction of all known factors with allowance for other possible factors can make

the solution of the origin of breast cancer in mice possible. All these factors play their part in the genesis of breast cancer in mice and it would be, at least, imprudent to state which is the most important.

SUMMARY.

Differences in quantity and/or quality of the milk factor derived from two different high-breast-cancer strains have been shown and referred to differences in the genetic constitution of these strains.

Large quantities of the milk factor can break down the resistance of mature susceptible mice and induce a high incidence of breast cancer in these mice, while similar amounts of the milk factor combined with forced breeding have failed to induce breast cancer in mature mice of a low-breast-cancer strain.

Adult mice of another low-breast-cancer strain, when given the milk factor, have failed to develop breast cancer, but have transmitted the factor and have induced a high incidence of breast tumours in susceptible mice.

The interaction of milk factor and genetic constitution and the presence of two sets of genetic factors, one controlling the susceptibility of mice to the milk factor, the other the propagation of the factor, both in high- and low-breast-cancer strains, is discussed.

The possibility of the development of breast tumours in susceptible mice without the milk factor has been shown.

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THE SIGNIFICANCE OF STUDIES WITH TRANSPLANTED TUMOURS.

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Most people would agree that studies with transplanted tumours have given information concerning the autonomy of malignant tumours and concerning their histogenesis. However, there is a tendency to regard such studies as somewhat old-fashioned and this field of study more or less closed. In this paper it is hoped to show that there is still valuable information to be gathered of significance for general biology as well as oncology.

General biology and tumour transplantation.

The genetical laws governing the transplantation of normal and malignant tissues are now generally accepted. In both cases a complex of dominant genes is concerned which Snell (1948) has called histocompatibility genes. There have always been two schools of thought concerning the nature of these genes, one that the genes determine iso-antigenic differences, the other that they determine "individual differentials"—substances with somewhat mysterious and ill-defined properties.

It is not easy to study these questions with normal tissues for technical reasons, one of which is that the number of factors is always high. With tumours the number varies, but may be low, and in addition tumours stimulate a better antibody response.

Iso-immune reactions are most easily studied in the erythrocytes, and it so happens that certain important iso-antigens in Strong's A strain of mice are shared by the erythrocytes and fixed tissues. The same is true of certain other strains, but the A strain is the easiest to work with. Four tumours from this strain have been studied genetically and serologically.

The first two were studied in England; in both it appeared that two histocompatibility genes were concerned, one of which was identical with a gene for an antigen known as antigen II. By serological means it was shown that the tumours contained another antigen in addition to II. Occasionally iso-antibodies were formed against it, but too irregularly and at too low a titre to be of value for genetical work (Gorer, 1937, 1938, 1942).

Those studied in America were both found to give single gene ratios in backcrosses. The importance of antigen II was again demonstrated (Table I). Furthermore, in conjunction with Snell and Lyman (Gorer, Snell and Lyman, 1948) it was possible to show close linkage between antigen II and the locus for "fused" (a tail anomaly). Full information is to be published elsewhere, but it can be seen from Table II that the linkage is close. We obtained very similar data with a dba tumour. The antigen concerned is closely related to II chemically, and for this reason we think it likely that the two genes are alleles which we have

TABLE I.—*The Influence of Antigen II on Tumour Inoculation.*

	Response to tumour. +	−	Total.
Antigen II present	28	3	31
Antigen II absent	1	37	38
Totals	29	40	69

TABLE II.—*Test for Linkage with Antigen II and Fused.*

Phenotype of mice.	Number of mice with— Antigen present.	Antigen absent.	Total.
Fused	0 (20)	37 (20)	37
Normal	37 (20)	5 (20)	42
Totals	37	42	79

NOTE.—The numbers in parentheses are the approximate number of mice expected in each class if the genes for antigenic constitution and "fused" or normal tails were on different chromosomes.

TABLE III.—*The Antigenic Similarity Between A and dba Red Cells.*

Tested on cells of—	Serum anti-15091a diluted 1 in— 2.	4.	8.	16.	32.	64.	128.	256.
A	+±	+	a.c.	a.c.	a.c.	+±±	+	−
dba-2	−	−	++	+++	++	+	−	−

Absorbed with cells of—	Serum tested on A cells after absorption (at 1/8). Dilution of serum. 8.	16.	32.	64.	128.	256.
A	−	−	−	−	−	−
dba-2	++	−	−	−	−	−

NOTE.—15091a is an A strain tumour. The sera were produced by inoculation of C57 blacks. The similarity is not shown to the same degree by all sera.

provisionally called H2 and H2[d] (Table III). Closely related antigens occur in strains CBA and C3H, so perhaps there is a long series of alleles at this locus. We are not certain how many cross-overs there were. Animals carrying the gene for fused may have normal tails, and there are other difficulties associated with tumour transplantation that will be discussed later on. In backcrosses involving 257 animals the recombinations were between 0 and 5 per cent.

Previous to these studies Strong (1929) had used tumour transplantation to show sex linkage of a histocompatibility gene, and Bittner (1933) found loose linkage with "dilution." The location of H2 was first found by Snell and Lyman using tumour inoculation without serological testing, and I hope I am betraying no confidences by saying that they have other instances of linkage as well. It will be seen that tumour inoculation is a most useful weapon in mapping chromosomes. Naturally it is best to use serological tests as well, but these may be

difficult. We may be certain that not all the pertinent antigens are shared by the transplanted tissues and the red cells—a fact that greatly increases technical difficulties.

Before discussing some of the pitfalls that may occur in detecting cross-overs by tumour transplantation it may not be amiss to call attention to another problem of interest to pure genetics, namely, polymorphism. In his discussion Ford (1945) alludes to the question of serological differences in man. I believe that all mammals (and perhaps many other forms) are polymorphic for histo-compatibility genes, of which the blood groups, etc., are special instances. I have examined a few wild mice all trapped in the same premises. Without going into details it may be said that they were not serologically uniform. Tumour transplantation indicates that this polymorphism may be maintained by a high mutation rate. Pure strains unless kept on a very small scale automatically crystallize into various sub-strains. It was shown by Bittner (1935) that a dba tumour would not take in all sub-strains, and this has been found to be a common finding, having been seen with various tumours arising in strains C57 black (Law, 1942; Gorer, unpublished) and C3H (Gross, 1947). The phenomenon could probably be found in all strains if sufficiently delicate methods are used. The reason for this is obscure. We have as yet no evidence that liability to disease is influenced by antigenic constitution, or indeed very little knowledge of the antigens' functions in the cell. Perhaps the polymorphism is connected with the risk of iso-immunization during pregnancy; this must be great where foetal absorption is common. If a species were completely uniform the risk would vanish, but it is unlikely that any species would be completely so over the whole of its range. On the other hand, if it were sufficiently polymorphic, the danger may be minimized. If a male has a rare antigen he would probably be heterozygous, and only half the litter would perish if iso-immunization took place. Further, in matings with different males, a female would be unlikely to receive successive doses of the same antigen. Lastly, it is possible that if a mixture of a large number of antigens is received, dangerous titres of antibodies are not formed against any one of them. Antigen II does not appear to be very mutable, but here mice also seem very polymorphic. Eight pure strains have been examined; all are somewhat different. As we have seen, there may be a large series of alleles here. The most powerful in this group ($H2^A$) is almost recessive to the allele in the C57 blacks, which is apparently non-antigenic. It is as though "O" in man was almost dominant over A or B—an interesting feature in view of Fisher's theory of dominance.

Transplantation and oncology.

To return more closely to the cancer problem, we may ask: If an A strain tumour gives a single gene ratio in crosses with C57 blacks, does this mean that they contain only one factor antigenic for the latter strain? The answer is in the negative. Both the tumours tested in Bar Harbor gave such a ratio, but it was shown conclusively that they both contained at least two antigens. This is easily explained immunologically. Antigens differ greatly in potency. Thus in man iso-immunization happens frequently against the classical Rhesus factor (R_o or D), but very seldom against antigen M. This doubtless explains the fact illustrated in Table I that mice with antigen II were resistant to tumour inoculation. If we were to breed from the 3 resistant young by crossing with a resistant

strain, we would get about 50 per cent susceptible progeny (MacDowell and Richter, 1932).

Errors of the above type are counterbalanced by precisely the opposite effect. Some tumours may grow and kill the host in spite of iso-antigenic differences. There is considerable indirect evidence of this, but recently it has been possible to show that an animal succumbing to a tumour derived from any other strain may form high titres of antibodies (Gorer, 1947). In many experiments these two sources of error usually seem to cancel themselves out, as the correspondence with Mendelian expectation is generally satisfactory. They may give rise to serious error in attempting to identify individuals as cross-overs or non-cross-overs, etc.

So far as the cancer problem itself is concerned, the latter of these two anomalies is by far the more interesting, since it helps to throw some light on the difference between normal and neoplastic cells and upon the effect of a neoplasm upon the individual bearing it.

It is now well known that tumours may "mutate." In general it may be said that tumours that have been transplanted a number of times appear to possess fewer antigens than they did at first. When first tested a tumour may appear to have upwards of 7 factors; later on, only one or two. Differences of this order cannot be accounted for by errors of the type just mentioned. There must be some real antigenic alteration. This might be due to mutations of dominants to recessive alleles. This is extremely improbable; one would have to have 6 or 7 homozygous mutations. A quantitative serological study of certain A strain tumours has given evidence that some antigens are greatly increased in amount. What may happen is that one or more antigens crowd the others out. If for the sake of argument we assume that antigen II occupies 10 per cent of the surface of a normal cell, in a malignant one it may occupy 90 per cent. These experiments have the disadvantage that we have no really satisfactory normal control. For example, we can show that the cells of a myeloblastic leukaemia contain increased amounts of antigen II, but we cannot get enough normal myeloblasts for comparison. There is evidence that analogous antigenic alterations may occur in human tumours. Zacho (1932) showed that the antigens M and N are present in increased amount in malignant tissues. With tumours of the alimentary canal one should be able to obtain sufficient normal mucosa for comparison. It is hoped during the coming year to study various antigens in this way.

We have already seen that the ability of a tumour to grow in an alien strain does not indicate complete loss of strain specific antigens. How, then, does it maintain itself? There is probably no simple answer to this question. Apparently the cells can maintain themselves in the presence of high titres of antibodies. Bacteria do the same thing in fatal cases of typhoid, streptococcal septicaemia, etc., although we do not know how.

In addition, there would appear to be some depressant effect upon the reticulo-endothelial system exerted to a lesser or greater degree by all malignant tumours Victor and Potter (1938) have shown that the metabolism of lymph nodes is depressed by inoculated leucotic cells, even though they have not yet become demonstrably invaded by them. Blumenthal (1942) has shown that animals with spontaneous mammary tumours are more susceptible than controls to implants of tumours from alien strains. Lastly, we have some evidence that

animals with transplanted (Browning, 1947) and induced tumours (Gorer, unpublished) are more susceptible to bacterial infection than are normal animals. One needs a great deal more information on this point, using pregnant animals, and animals with grafts of embryonic tissues as controls. This may turn out to be an important property of malignant growths. At present the bulk of expert opinion is against invoking any direct toxic effect of tumours to account for the illness one observes in human cancer. This is quite logical. There are numerous reasons why a cancer patient should be ill. However, work with transplanted tumours suggests that the subject cannot be considered closed.

SUMMARY.

1. Tumour transplantation was used to show the antigenic basis of transplantation immunity in general.

2. One of the pertinent histocompatibility genes has been shown to be closely linked to the gene for a tail anomaly in mice.

3. It is believed that all mammals (and perhaps many other forms) are highly polymorphic for histocompatibility genes. There is evidence that some of these genes have a high mutation rate in mice. The possible significance of this is discussed in the text.

4. Serological studies show that tumours may kill an animal in spite of antigenic differences. Some antigens are weak, and rarely elicit an effective defensive reaction. Genetic studies on transplantation are therefore not a completely accurate indication of antigenic structure.

5. Tumours undergo some antigenic simplification during transplantation. Probably one or two antigens increase in amount and crowd out the others.

6. Malignant tumours appear to exert a depressive action on the defences of the host.

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GENETIC STUDIES ON LEUKAEMIA IN MICE.

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In the last two decades the heredity of leukaemia in mice has been the object of fairly extensive investigations, which, however, have not fully solved the problem. Summing up the results, it may be said that there is an incompletely dominant inheritance, and that environmental factors play a varying part in the development of the disease within the different strains.

The experiments which form the basis of this paper do not technically differ much from previous examinations, but our material shows certain details which deserve special mention.

In order to study the inheritance of leukaemia in mice we have made crosses between Aka line mice and B line mice, and examined the offspring of the F1, F2, and the backcross generations to both the original strains.

The Aka line is a branch of Furth's well-known Ak line. Through many generations it has been inbred, and consequently can be considered as approximately homozygous. Sixty-three per cent of the animals within this strain die from leukaemia, and Fig. 1 illustrates the age distribution of animals dying of leukaemia in this line. The average age of death of Aka mice with leukaemia is 295 ± 5 days. For the F1 it is $591 \pm 19{\cdot}6$ days. Almost all cases of leukaemia in the Aka line are lymphogenous or of a very immature type—the so-called stem-cell leukaemia. Myelogenous leukaemia is found, but very seldom.

The low leukaemia line which we have used for the cross-breeding is called B. This line is also inbred. Among 113 B mice observed during this experiment, no case of leukaemia or sarcoma was observed. Among hundreds of B mice we have previously observed, only one case of lymphogenous leukaemia was found.

In the F1 there was found 43 per cent of deaths due to leukaemia; the age distribution of these mice dying from leukaemia is shown in Fig. 1. The age distribution is somewhat wider than in Aka mice and the peak is displaced about 300 days later, so that in this case the average lifetime for mice dying from leukaemia is $591 \pm 19{\cdot}6$ days.

In the F2 we encountered 37 per cent cases of leukaemia, and the distribution of age appears to be very wide, as shown by Fig. 2. There were many early cases, but also a good many late ones. The curve may be regarded as a combination of two distributions, one corresponding to the Aka mice and one corresponding to the F1.

The backcross derived from the F1 crossed with Aka shows a rise in the frequency of leukaemia to 49 per cent, and the curve concerning the distribution of age shows, as is seen from Fig. 3, an increased number of early cases compared with the F1 shown in Fig. 2. This backcross curve is even more distinctly bimodal.

The offspring derived from backcrossing the F1 to the B line gave 28 per cent cases of leukaemia. As the curve at Fig. 4 shows, the cases are mostly late.

The form of the curve is, however, somewhat irregular, owing to the rather low number of mice. If, on the basis of these facts, we are going to discuss the inheritance of leukaemia in mice, we will at first have to consider the frequency of leukaemia within the different generations, as it is summarized in Table I.

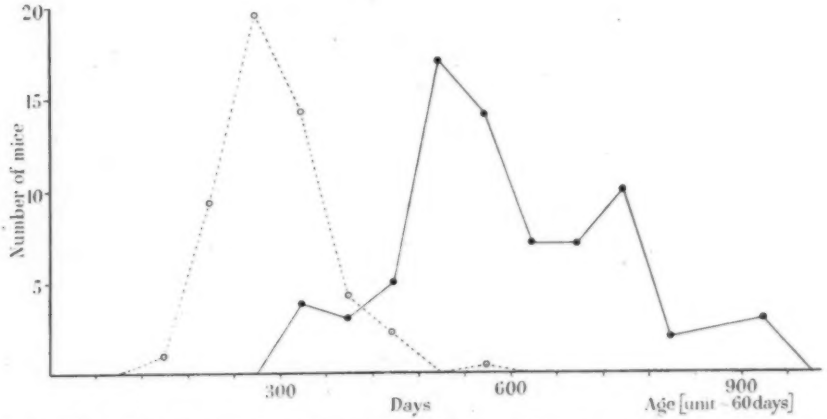

FIG. 1.—The age distribution of mice dying from leukaemia within the Aka line (- - - - -) and the F1 (———).

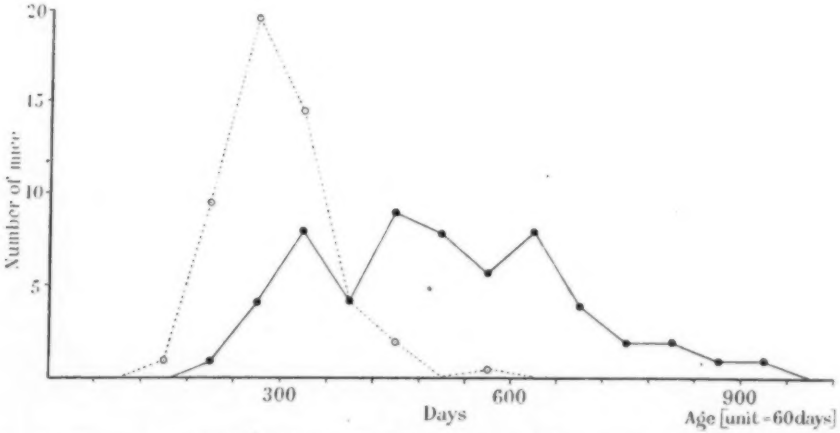

FIG. 2.—The curve for the Aka mice (- - - -) is drawn as well for comparison. The curve for the F2 shows a tendency to a bimodal form.

You will see that, in spite of the genotypical uniformity of the mice of the Aka line and the F1, leukaemia only develops in respectively 63 per cent and 43 per cent of the animals. As MacDowell and Richter (1935) have indicated, this is owing to the fact that environmental factors as well as genetic ones play

a part in incidence of the disease, but it is questionable whether these factors influence the development of the disease as such. The percentages mentioned are—as is the case with the figures from previous experiments—calculated by comparing the number of mice dying from leukaemia with the total number of

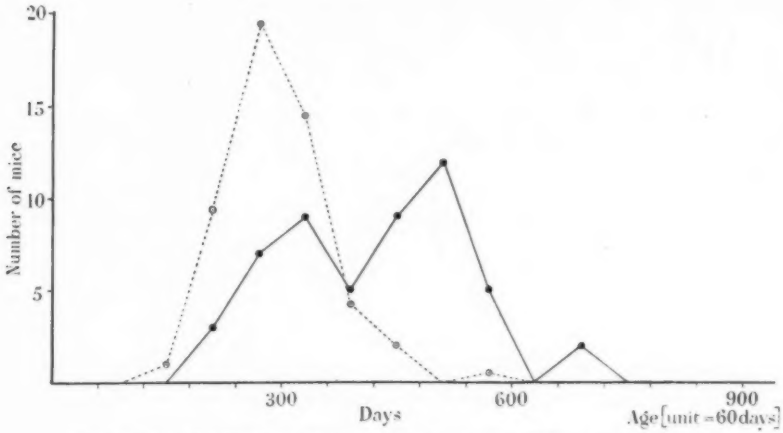

FIG. 3.—Age distribution of leukaemia in backcross of F1 mice to Aka line.

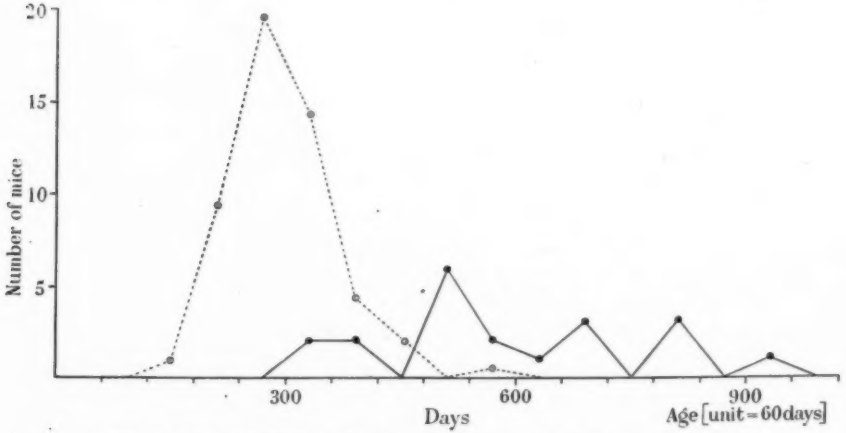

FIG. 4.—Age distribution of leukaemia in backcross of F1 mice to B line.

dead mice, the animals dying before the first case of leukaemia occurs not being included.

It is quite evident that this mode of calculation is entirely inadequate, because deaths caused by other reasons than leukaemia (such as intercurrent diseases), will influence the result to a very high degree. The actual risk of getting leukaemia

for the mice within the different generations will therefore be considerably greater than the percentage incidence shows.

Providing the B line is free from genes for leukaemia, the presence of 43 per cent cases in F1 shows that the inheritance is dominant. The frequency in the F1 is not so great as in the Aka line, but this can be in part due to the fact that the cases appear at an average age of 300 days later, so that the deaths from other causes will decrease the frequency of leukaemia.

The figures found in this material, as seen from the following considerations, support the view that the inheritance may depend on one single dominant gene. If we suppose that this is the case, we can calculate the expected numbers of leukaemic animals in the F2 and backcrosses to the parent lines on the basis of the observations in the Aka and the F1 mice. These results are calculated in Table I, taking into account the deaths from other causes. These figures agree very well with the observations, but in the calculated mortality curves the agreement was not so evident. But the statistical evaluation of these curves is so complicated that it is doubtful whether too much weight can be laid upon the calculated curves.

TABLE I.—*Frequency of Leukaemia.*

	Strain of mice.					
	Aka.	B.	Fl.	F2.	BC. F1 × Aka.	BC. F1 × B.
Number of mice in the experiment	368	113	322	249	153	145
Number of mice surviving the first case of leukaemia	322	113	167	156	106	78
Number of mice dying from leukaemia (observed)	203 =63%	0	72 =43%	58 =37%	52 =49%	22 =28%
Number of mice dying from leukaemia (calculated)	—	—	—	62·4	59·5	16·4

The backcross generation F1 × B gave 28 per cent or 22 cases of leukaemia. An inheritance depending on one dominant gene will by calculation give 16·4 cases. The difference is not significant. If we assume the presence of two genes the calculated number will only be 8·2. These data thus support the hypothesis of a single dominant gene.

The variation in the average lifetime of the leukaemic animals has previously been described by MacDowell, Potter and Taylor (1945), MacDowell and Richter (1935), Furth and Barnes (1941), Kirschbaum (1944), and Cole and Furth (1941) in similar crossbreeding experiments. The phenomenon seems therefore to be independent of the character of the lines used, which fact seems to refute the previous supposition that casual changes in the average lifetime of all the mice may influence the result.

I would sooner say that the discovery confirms the supposition that the immature leukaemic cells arise by a somatic mutation in cells which possess a labile

gene (or a complex of genes). Where and how this mutation arises could be a matter of chance. If the animals are homozygous with regard to the labile gene, the mutation will naturally arise earlier and more frequently than is the case if the animals are only heterozygous. The latter case seems to show that it is not the predisposition itself for the disease which is inherited, but the tendency for a somatic mutation leading to the development of the disease.

MacDowell and Richter (1935) were of the opinion that their material showed the presence of a maternal factor, because the offspring of a cross between females from the leukaemic strain and males from the resistant strain, both in the F1 and F2 as well as in the backcrosses to the original strains, showed more frequent cases of leukaemia than in the reciprocal crosses. Other investigators have not been able to obtain a decisive difference in reciprocal crosses. In my material there is a similar difference in the F1, the offspring of the cross B-male × Aka-female giving a leukaemia frequency of 46 per cent, while the reciprocal cross gives only 34 per cent. However, it appears that in the F2 the result is contrary. Consequently it is most probably not maternal factors with direct influence on the development of leukaemia which have determined this variation, but a variation in the number of deaths from intercurrent diseases that has influenced the frequency. In other words, here again it is the estimation of the risk of disease which is inadequate. It is difficult to say whether the same is the case with MacDowell and Richter's (1935) material.

Finally I should like to mention that in the hybrids there was a considerably greater morphological variation between the cases of leukaemia than in the original inbred Aka line. In the F1 and F2 myelogenous leukaemia is more frequent.

In the Aka line the disease very frequently manifests itself only as a colossal thymus tumour. Table II shows the frequency of simple thymus tumour in the

TABLE II.—*Frequency of Simple Thymus Tumour.*

	Strain of mice.					
	Aka.	B.	F1.	F2.	F1 × Aka.	F1 × B.
Number of cases examined	200	0	56	48	44	14
Enlargement of thymus, %	28	0	7·1	10·4	25	0

leukaemias of the lines and crosses studied. It indicates that the simple thymic tumour is especially prevalent in types of mice which also show most cases of early leukaemia.

Transplanted leukaemia.

As well as these experiments on spontaneous leukaemia, we have also examined the inheritance of susceptibility to transplanted leukaemia in the same hybrids from the crosses of Aka and B.

In this case our technique diverged to some degree from the usual technique, as we did not use a particular line of leucotic cells. On the contrary we have in all the experiments used new cases of lymphogenous leukaemia from Aka mice for transplants.

We chose this method in order to maintain the relation between the transplant and the host as specific as possible, it being a well-known fact that passage causes

the leucotic cells to undergo changes which are reflected in changes in the survival of the transplanted animals, the size of the cells, and the susceptibility of the mice used in the experiments (MacDowell, 1945). These changes always go in the direction of less specificity.

By this experimental arrangement we were prevented from detecting the result of the segregation in the F2 and the BC (F1 × B), because the different transplants may have been genetically different ; but on the other hand, we have been able to study the mode of growth of the transplant in the different genetic environments under the most specific conditions.

In every experiment we have as far as possible transplanted simultaneously litters of mice from the Aka line, B line and the F1, F2 and backcrosses to the parent strains with leucotic tissue minced, suspended in Ringer's solution, and injected intraperitoneally. Table III shows the result of these experiments.

TABLE III.—*Type of Leukaemia Encountered in Transplants.*

	Strain of mice.					
	Aka.	B.	F1.	F2.	BC — Aka.	BC — B.
Number of mice	300	101	46	232	130	100
Positive, %	100	0	100	55	100	10
Generalized growth	100	0	100	57	100	10
Intermediate forms	0	0	0	20·3	0	30
Tumour-like growth	0	0	0	22·7	0	60

In the Aka strain 100 per cent takes are found, which all give the pathological picture of a generalized leukaemia. The same is found in all the F1 and the backcross from the F1 to the Aka. In the F2 only 55 per cent give leukaemia, and out of these 55 per cent, 57 per cent are identical with the cases in the Aka strain, while 22·7 per cent show only a localized growth at the place of transplantation. In 20·3 per cent an intermediate state between these extremes is found. In the backcrossing to the B (F1 × B) only 10 per cent takes are found, out of which 60 per cent are localized, while 30 per cent are intermediate, that is to say, a tumour-like growth occurs at the place of transplantation accompanied by generalized changes. Only 10 per cent are generalized.

The question is now, what is the cause of this variation ? Does it reveal some rules concerning the growth and diffusion of the tumour cells, or is it only a phenomenon dependent on a greater or smaller resistance in the hosts ? At least we may draw the conclusion that the problem of the inheritance of susceptibility to transplanted leukaemia in this particular cross is more complicated than the case has been with other neoplasms.

We have tried to find out whether there are cytological differences between the cells in the localized and in the generalized cases. In some cases we found larger cells in the tumours. However, these examinations are not yet complete, and I would prefer not to give a definite statement as yet.

SUMMARY

A brief account is given of studies on the frequency and age distribution of mice that died from leukaemia in the different generations of a cross between the Aka line of mice with 63 per cent leukaemia and the resistant B strain.

The curves and figures support the view that the inheritance of the disease in this cross is dependent on one single, dominant gene.

Transplantation experiments show that the resulting disease has features that are in part dependent upon hereditary factors.

The studies here presented were carried out with the aid of grants from Anders Hasselbalchs Leukaemifond and from the Lady Tata Memorial Trust.

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MUTATIONS INDUCED BY CARCINOGENS.

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From the Carnegie Institution, Cold Spring Harbor, New York.

Given at the Symposium on the Genetics of Cancer, London, June 24 and 25, 1948.

USING Drosophila as experimental material, a large-scale survey of mutagenic potency in carcinogens and chemically related non-carcinogenic compounds was begun in June, 1947, just a little over a year ago. Although Drosophila is a fast breeder, so that more than thirty generations can be raised during a twelve-month period, one year is still too short a time to accomplish much in a new field of research. This is particularly true when in the course of the work unexpected complications arise, requiring effort along lines that branch away from the main problem, as happened in our case. We spent more than eight months working on one such incidental problem, which had to be solved before we could proceed with the main work. This effort was well rewarded, however. We not only improved our experimental approach, but also acquired information that will further our understanding of the intricate role played by biological factors in regulating the influence of external agencies on living cells.

In view of all this, I should like to point out that our investigations of the genetic potencies of carcinogens are still in an early stage. The main outlines of the structure on which conclusions may be based are already evident; but details are still missing, and it will be some time before they are filled in.

My principal interest is the study of spontaneous and induced mutability, and on several occasions during the past fifteen years I have made attempts to

induce mutations by means of various chemicals. In one such attempt Drosophila were raised on food to which chemicals had been added ; in another, chemicals were injected into larvae and flies ; in a third, young gonads were removed from larvae, immersed in solutions of chemicals, and injected into other larvae. The results were negative in each case. Therefore I was very much interested in the results obtained by Auerbach and Robson when they induced mutations in the sperm of Drosophila males by exposing them to vapours of various mustard compounds. I was particularly glad to learn that vapours, presumably entering through the spiracles of the flies, were able to reach the testes and affect the sperm. At the time when the reports of Auerbach and Robson's success reached us we were engaged in an extensive war research project dealing with the generation and properties of fine mists (aerosols). When I learned, therefore, that vapours could reach the sperm of Drosophila I was confident that it would be possible to produce fine aerosols of chemical solutions that could do the same thing. It was evident that, if we could develop an aerosol method of inducing mutations, we could work with a wide selection of chemicals that are soluble in solvents not injurious to the flies, and would not be limited to chemicals that can be vaporized at temperatures not lethal to the flies. After much experimentation we finally succeeded in developing an aerosol technique that is effective in inducing mutations in Drosophila males.

The method is very simple. Males are kept for several hours (from 6 to 200) in an atmosphere containing an aerosol of the desired solution ; and after this treatment their sperm is tested for induced genetic changes by standard genetical methods. The flies are treated in a milk bottle, and the aerosol is generated intermittently, for 30 seconds every 30 minutes, by a De Vilbiss nebulizer.

In our work with chemicals we have concentrated on carcinogens, because there is a feeling among geneticists that these compounds should also be mutagenic. What classifies them as carcinogens is their ability to change normal cells into cancerous cells—that is, to produce permanent changes in living cells, which are transmitted to the progeny of those cells. This definition can also be applied to mutagens, which cause permanent and heritable changes, or mutations, in cells.

This series of experiments with carcinogens also included chemically related non-carcinogens. Tests were made with nine polycyclic hydrocarbons (four carcinogens and five non-carcinogens) and with seven azo compounds (three of them carcinogenic). The observations were made on induction of X-chromosome lethals and of chromosomal aberrations coincident with lethals. Conclusions reached on the basis of the experimental results are summarized in Table I.

From Table I a definite correlation between mutagenicity and carcinogenicity is evident. Of the seven carcinogens tested, six were found to be mutagenic ; and of the nine non-carcinogens only two were found to be mutagens, and one is still classified as doubtful.

The evidence now available suggests that some chemicals (dibenzanthracene, benzpyrene, and hydroxyazobenzene) induce both gene changes—i.e. lethals—and chromosomal aberrations, whereas some others (benzanthracene, dimethylaminoazobenzene, and 2-amino-5-azobenzene) are more effective in inducing chromosomal aberrations.

During our experimentation it was observed that different males treated at the same time quite frequently showed different results ; that is, genetic changes could be induced in some males more readily than in others. Table II gives the

TABLE I.—*Conclusions from Experiments to Test Mutagenic Potency of Carcinogens and Chemically Related Compounds.*

Mutagenic.		Non-mutagenic.
Cyclic hydrocarbons :		
1,2,5,6-dibenzanthracene	L–CA	anthracene
20-methylcholanthrene	L	phenanthrene
3,4-benzpyrene	L–CA	pyrene
1,2-benzanthracene	CA	alpha-naphthylamine
		beta-naphthylamine
Azo compounds :		
p-hydroxyazobenzene	L–CA	azoxybenzene
p-aminoazobenzene	L	*p*-diethylaminoazobenzene
p-dimethylaminoazobenzene	CA	Doubtful.
2-amino-5-azobenzene	CA	azobenzene

——— = carcinogenic. Mutagenic effect observed through L = lethals ; CA = chromosomal aberrations.

TABLE II.—*Number of X-Chromosome Lethals Found among Males Exposed to 1,2,5,6-Dibenzanthracene Aerosol during One Experiment (No. 118).*

Male number.	Sperm tested.	Number of lethals.	Male number.	Sperm tested.	Number of lethals.
1	78	0	10	104	0
2	71	0	11	118	2
3	81	0	12	63	1
4	92	0	13	51	0
5	61	1	14	61	2
6	43	1	15	84	1
7	55	1	16	49	1
8	134	1	17	25	4
9	101	1			
			Total	1271	16

P = <0·0001.

data from one experiment in which such differences were observed. It was noticed also that similar treatment in different experiments occasionally brought about different results (Table III). We spent a considerable amount of time

TABLE III.—*Data Showing Variations between Effects Obtained in Different Experiments in which Similar Treatment with 1,2,5,6-Dibenzanthracene was given.*

Experiment number.	Number of males tested.	Number of sperm tested.		
		Total.	Lethals.	%
185	20	1011	34	3·36
225	16	1235	2	0·16
245*a*	16	781	6	0·77
245*b*	24	805	3	0·37

P = <0·0001

and effort in tracing down the cause of these differences, investigating various environmental and genetic factors that might form the basis of variability. At present the most likely solution seems to be that a gene is responsible for the observed variability of effect between different males and between different experiments.

I shall conclude this presentation by quoting freely the conclusions given in a paper presented at the meeting of the Fourth International Cancer Research Congress in St. Louis last September.

The results of our experiments show a close correlation between the mutagenic and carcinogenic properties in certain chemicals. From these results it seems reasonable to infer a common causative mechanism relating mutagenicity and carcinogenicity. This inference is further strengthened by the behaviour of all known non-chemical carcinogens, such as X-rays and related radiations, ultraviolet rays, and heat—all of which are also mutagenic. The most obvious and probable relation between mutagenicity and carcinogenicity is the one suggested by the hypothesis that cancer may originate through a gene mutation occurring in a somatic cell. Such a cell, and the cells derived from it by division, would have their properties changed so that they would behave as cancerous. This would mean that higher organisms possess a gene—or, more likely, a number of genes—whose mutations can initiate a cancer-type cell. It may be assumed that such mutations, like a great majority of mutations in other genes, would occur spontaneously with a very low frequency. The human body has a tremendously large number of cells, however, so that it is probable that a cancer-type mutation would occur a number of times among the cells of an individual. Not all of these need give rise to cancer, since a large proportion of the cancer-type cells might be prevented by normal cells from dividing, or might be eliminated in some other way.

If gene mutation is responsible for the origin of cancer, then all mutagenic agents may be expected to increase the frequency with which such mutation occurs, and consequently to act as carcinogens. In our fight against cancer, therefore, precautions should be taken to avoid exposure to all mutagens—chemicals as well as radiations. Such precautions, however, even if rigorously enforced, would only lower the incidence of cancer; they could not entirely prevent its occurrence. There would still be a chance left for cancer-type mutations to occur among the billions of cells that constitute the human body, and a mutated cell that continued to divide would give rise to cancerous growth. We know that mutations do occur with great regularity, caused by some force not yet explained, and that we have no means of stopping or controlling their occurrence. Consequently, if cancer originates through a genetic change, our chances of finding ways to prevent it are very, very slight. We should be able to reduce its frequency by avoiding contact with carcinogenic and mutagenic agents; and in this effort a more extensive knowledge of the mutagenic capacities of various substances should be a valuable asset.

This investigation was aided by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council. Experiments were carried on in collaboration with Dr. Bruce Wallace, and with the assistance of Mrs. Jennie S. Buchanan, Mr. Peter A. Peterson, Miss Sara L. Rohrer and Miss Helen S. Spring.

THE PLASMAGENE THEORY OF THE ORIGIN OF CANCER.

C. D. DARLINGTON.

From the John Innes Horticultural Institution, Merton.

Given at the Symposium on the Genetics of Cancer, London, June 24 and 25, 1948.

1. *The cell analysis.*

Cancer stands at what might be the meeting-place, but what is in fact the no man's land, between the three disciplines of heredity, development, and infection. Its very existence leads to a contradiction between our fundamental notions in these three fields. For this reason we have usually shrunk from asking the crucial questions it puts to us, questions which were indeed, until now, too fundamental to be answered. I must therefore ask those who wish to follow me to be prepared to surrender certain established habits of reasoning, at least for the duration of the journey.

The first problem of cancer is to discover the general principle of its origin, whether in terms of an agent or a process. We are probably all agreed that tumours, although usually, or perhaps always, preceded by abnormal local conditions, arise by a sudden change. This change consists in an increase in the rate of growth of a cell or a group of cells. In this way there arises a new cell-lineage distinct from its antecedents, and one which can propagate its new property irreversibly and even indefinitely. It has the character of a genetic change, a somatic mutation. Secondary changes may follow establishing subsidiary cell-lineages, some involving further increases of growth-rate and de-differentiation, and all surviving and multiplying subject to natural selection. To these secondary changes I shall return later.

The chemical conditions arising in the changed tissue have been examined by Santesson and Caspersson (1942), while the processes of its cell division have been described by Koller (1947*a*) for epithelial tumours and by La Cour (1944) for pernicious anaemia, which is an analogous condition depending on the enhanced multiplication of the red blood precursor cells of the bone marrow. The conditions and processes fit together. An excess of ribose nucleic acid in the cytoplasm seems to determine more rapid protein production, and more rapid division of cells and nuclei. The rapid nuclear division, as usual in ordinary tissues, reveals chromosomes overcharged with desoxyribose nucleic acid and often improperly co-ordinated with the spindle, so that polyploid as well as deficient cells (with as few as 32 chromosomes) are formed and breakage of chromosomes is also frequent. These errors are no doubt aggravated by the accumulation of lactic acid which arises from the deteriorating conditions of respiration (Thomas, 1945).

The development of unbalanced nuclei in tumours is without precedent in any living tissue. It implies a relaxation of detailed control in the nucleus which is also without precedent. And this in turn argues that the nucleus is not itself directly responsible for what is going on.

Similar behaviour to that of cancer-cells has been observed to arise in plants from the action of particular chromosomes or of nuclear genes. A single polymitotic gene in maize and extra heterochromatic chromosomes in millet both cause rapid and unwanted nuclear divisions in the pollen grain. In maize up to four extra mitoses take place before the chromosomes have had time to divide immediately after meiosis. The nucleus is forced into division, and the unsplit chromosomes are scattered on a spindle which merely disperses them into a number of deficient nuclei, each of which is again compelled to divide before it is ready, and in this way the whole pollen grain is brought to ruin. In millet the mitoses (also up to the number of four) do not occur before the chromosomes are split, but the whole pollen grain is none the less consumed and killed in the end by the production of redundant nuclei. The pollen grain is thus, in a sense, an encapsulated tumour (Darlington, 1947).

Comparison of these cases of polymitosis in plants with animal tumours enables us to clear our minds on one point. Polymitosis is not due to a somatic mutation. It is a property of the whole organism. It affects every cell of a particular type in the body. And it acts as soon as that cell arises. The spontaneous tumour on the other hand arises at random both in time and space. It does not affect all cells of one type, but only one cell, nor is it the first cell of a particular normal type to arise which is affected. We are therefore confirmed in regarding the spontaneous tumour as due to a genetic change.

This conclusion removes the contradiction between development on one side and heredity and infection on the other by withdrawing to a lower level of analysis. The organism is now seen from the cell point of view as a vegetatively propagating colony. The cell is the individual having its own hereditary lineage. So long as the normal course of development is followed it is assumed to propagate itself without change. When a cell in such a colony diverges it does so as a result of a process or agency which may fall into two possible categories: mutation or infection.

2. *Mutation and infection.*

Now, when we consider the range of types and conditions of cancer we find that a multiplicity of organisms, tissues and agents is concerned. In this range of variation two differences are significant for our present purpose: that between spontaneous and induced, and that between infectious and non-infectious, cancer.

Cancer, indistinguishable from the natural kinds and in a great variety of kinds, can be produced by chemical agents, the carcinogens. The experimental evidence bears out the *prima facie* case for mutation. How far does it enable us to distinguish between nucleus and cytoplasm as the seat of the mutation? In general the most efficient carcinogens (such as the hydrocarbons) and the most efficient agents of nuclear mutation (such as mustard gas) do not coincide. Carcinogens do not damage the nucleus in proportion to their effect as carcinogens (Darlington and Koller, 1947). Thus the nucleus again seems to be excluded, just as it is by the direct evidence. But the evidence of X-ray effects is a more serious objection to a nuclear origin. X-ray damage leads to the development of cancer only when the dose has been so heavy as to damage the cytoplasm, and only then after prolonged delay. Light doses which have a proportionate effect on the breakage of the chromosomes and the mutation of the

genes do not induce cancer at all. Here then the evidence begins to turn decisively to the cytoplasm as the source of the change—the only organ to which we can look for the carrying of the cancer mutation.

It is, however, when we come to the distinction between the infectious and the non-infectious types of cancer that we can clinch the argument. This distinction has in the past led to confusion owing to the high professional status but dubious credentials of the word *virus*, a word which has inevitably been used to describe any cancer-producing agent with a capacity for invasion. Perhaps I may be allowed to examine this status and these credentials, and to make use in doing so of the underworld of plants and protozoa which are not usually thought relevant to the cancer problem.

3. *Plasmagenes and proviruses.*

In the first place, self-propagating particles transmitted by heredity, but lying outside the nucleus, are now known in all the chief groups of organisms—

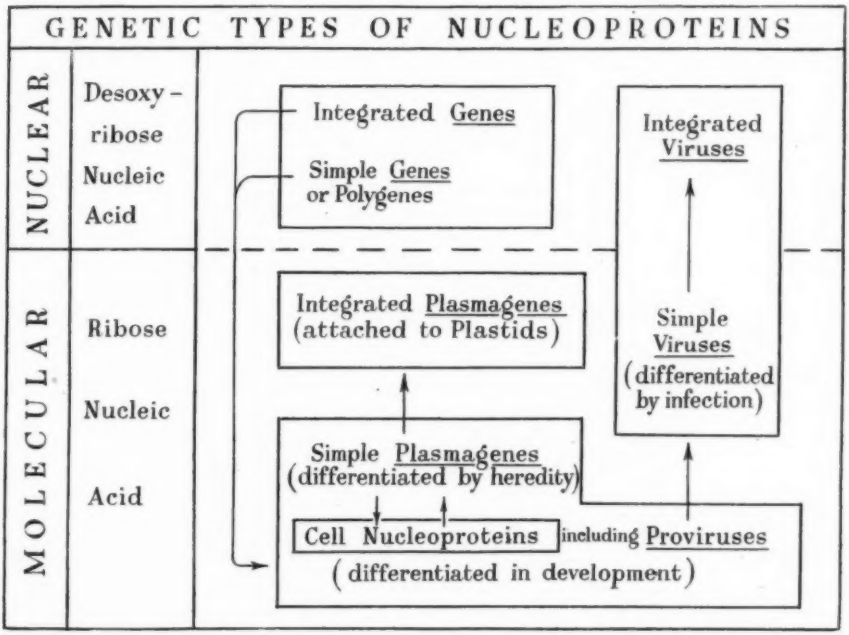

FIG. 1.

first and foremost in plants and protista, but recently also as high as the insects. These particles, which are known as plasmagenes, sometimes have a long independent history in the cytoplasm, and sometimes seem to be derived recently from the products of the nucleus (Fig. 1). Their number in the cell can be

estimated, and undoubtedly varies for each type of plasmagene under different conditions, in different tissues and in different stages of development.

Now plasmagenes, like all other such determinants, are susceptible to mutation. Certain of them control the formation of plastids in plant cells. It is not possible to relate here (as I have elsewhere) the long detective story of investigations which have led in the course of 40 years to the revelation of the character and continuity of the plastid plasmagene. Suffice it to say that, like the centromere and nucleolar organizer of the chromosomes, it carries its stock-in-trade on its back, which being a sac of green pigment marks or tags its owner and enables us to count its numbers in the cell and note its changes. It can mutate from green to white and *vice versa*, so that cells can occur with mixed green and white plasmagenes ; and, moreover, the same plastid may be starved white, as it were, by a stepmother nucleus, and recover its normal colour when it is put back with its own proper parent.

Finally, plasmagenes are capable of infection. Prolonged coitus causes a *Paramecium* to be infected by the Kappa plasmagene of its mate. Transplantation of an organ or of serum from a CO_2-sensitive *Drosophila* infects a non-sensitive fly, including its egg, with the sensitive plasmagene (Sonneborn, 1948 ; L'Héritier, 1948).

These infections, it will be seen, are artificial, or at least unnatural. Now the distinction between natural and artificial infection has long been known, although little regarded, in the discussion of plant viruses. A number of aberrant conditions can be transmitted from stock to scion, and some even have arisen in a scion after it has been grafted on a healthy stock. These are artificial diseases ; they are not transmitted in nature, but only by grafting. Some may have arisen by the mutation of self-propagating proteins in the cells of plants propagated over long periods by vegetative means (as tumours can be). Others have certainly arisen by the migration or transplantation of proteins from one organism to another. In either case they have a property of infection which they can reveal only in artificial circumstances.

Thus there are self-propagating proteins in the cells of plants and animals which are normally transmitted by heredity, but have the properties necessary for natural infection if an infective agent or vector were to come along. And no doubt such an agent often has come along to set such particles on the new track of infection. As we find them, however, they are not specialized for natural infection by the selection and adaptation of their ancestors. We make a great mistake therefore in calling them viruses : they are *proviruses* (Darlington and Mather, 1949).

Conversely it has been known for 40 years that the virus *Rickettsia* is carried by the eggs of its vector *Dermatocentor*. The terror of its *infection* for man has closed our eyes to the fact of its *inheritance* for the tick. What appears as a virus in one host is indistinguishable from a plasmagene in the other—and it may well be that the plasmagene is the older (L'Héritier, 1948).

At the same time Billingham and Medawar (1948) have shown that a self-propagating particle differentiated in development may by its diffusibility enjoy certain of the properties of infection—a situation which can come to our notice only when the particle is as innocuous, and the differentiation as trivial, as the black pigment producer of a piebald mammal. Differentiation, like heredity, excludes infection, but only in things that matter and things that have a future.

4. *The necessary hypotheses.*

With these principles to guide us, we may turn back to the cancer-producing agents. We see that some have the properties of plasmagenes, some of proviruses, and a few of true viruses. They have arisen in different ways.

The plasmagene and provirus types must have arisen by mutation of self-propagating particles in the cytoplasm of the first cells to have been affected by them. This means that they have arisen from previously innocuous plasmagenes. And not merely from plasmagenes of the same species that we find them in, but actually of the same individual. They have never been guilty of infection, and are therefore totally unadapted for or against it.

The virus type may well have arisen likewise by mutation of a plasmagene, but it must have been a mutation followed by others adapting it to infection. Moreover, this type falls into two groups by a most instructive distinction. The wart in man or Shope papilloma in the rabbit are generally contagious, and may therefore have arisen anywhere. The milk-agent, on the other hand, cannot be imagined as having arisen anywhere save in the mouse itself, in which it is inherited by the milk instead of by the eggs.

How accidental is the distinction between the infectious and the non-infectious particle is revealed most strikingly by one true virus. The Shope papilloma is naturally infectious in the cottontail rabbit, and it can be successfully inoculated into the domestic rabbit as a cell-free extract. But in its new host it not only ceases to be infectious ; it even ceases to be inoculable. It loses the property of infection, and becomes transferable, if at all, only in whole cells. This is just the opposite transformation to the one by which proviruses arise or demonstrate their existence when two plants are grafted together. The capacity for infection which distinguishes between proviruses and other plasmagenes is therefore conditional. It depends on the relationship between the self-propagating protein and the type of cell in which it is propagating itself.

Thus the range of behaviour of the cancer determinants between infection and non-infection is as wide as that of the other types of self-propagating body in the cytoplasm. The two groups of agents correspond in nearly every respect (Fig. 2).

There can be no doubt therefore that the cancer determinants arise as mutant particles in the cytoplasm—that is, as plasmagenes.

5. *Objections and difficulties.*

Three grounds for suspicion of the plasmagene origin of cancer are now worth discussing. First, the cancer agents have one characteristic which will at once be noticed as marking them off from all previous plasmagenes. They are not transmitted in heredity. The reason, however, is not far to seek. Any cell in the germ-track which develops the cancer potentiality will be automatically put out of the running. It will be sterilized. Now the inheritance of lethal mutations in the nucleus, when they are recessive in their lethality, is mendelian and can therefore be followed in experiment. But cytoplasmic mutations are non-mendelian ; they know of no dominance or recessiveness. If they are lethal they destroy their host and themselves, and that is what happens to the cancer mutation.

	TRANSMISSION	PLANT	ANIMAL	
PLASMAGENES	By whole cells only No invasion of cells by particles	Rogue peas and tomatoes Strawberry yellows	Most mammalian tumours spontaneous and induced (cells invade, particles do not)	ARISING BY MUTATION
PROVIRUSES	By cell-free extracts or invading particles Artificial conditions	K. Edward potato L. Lambourne apple Graft-given variegation	Rous sarcoma (fowl) CO_2-sensitive (Drosophila) [Killer (Paramecium)]	
VIRUSES	By natural infection direct or through vectors	Insect — carried agents	Milk-carried Cancer (mouse) Shope Papilloma (cottontail rabbit) Warts (man)	
		ARISING BY TRANSPLANTATION		

FIG. 2.—Orders of transmission of proteins and modes of origin of viruses.

Secondly, it may be asked, why, if tumours are due to plasmagene mutations, other such mutations do not occur in our bodies having other recognizable effects. The answer is that no mutation can express itself in a mature cell except by renewing the growth which has ceased.

Finally, it may seem odd that a condition induced by cytoplasmic mutation should be cured by radiations acting specifically on the nucleus. Koller (1947*c*) has shown that radium and X-rays act on tumour cells with high efficiency by breaking the chromosomes. A whole tissue can be destroyed by rendering its nuclei unworkable in this way. There is, however, no contradiction between the assumptions of a cytoplasmic cause and a nuclear cure. The nucleus is the only wheel in the cell that we can put a spoke in. That is especially easy to do in rapidly dividing cells (Darlington and La Cour, 1945). We are therefore merely attacking the malignant cell where it is weakest.

Incidentally the effect of irradiation in breaking the chromosomes and stopping the growth of the cells leaves no doubt that the remarkable deficient nuclei found in tumours by Koller (1947*b*) will have a limited life. They must be continually thrown off by sticky chromosomes and multipolar spindles, only to peter out as soon as the barriers to inter-nuclear co-operation have grown up between them and their fully provided neighbours.

One more question is worth answering: What form would the mutant protein be likely to take in the tumour cell? On account of its rapid multiplication it might well show a higher degree of aggregation than its progenitor. It would then appear as an alien particle in the mutant cell. This is borne out by the electron microscope observations on two chicken tumour agents of provirus type by Claude, Porter and Pickels (1947).

6. *The particle analysis : competitive propagation.*

The cancer mutation, as we saw, is often followed by other changes beyond the primary one of change in growth rate. These may be described under the headings of *dedifferentiation* or the loss of tissue character, *metastasis* or migration, secondary mutation and various breakdowns of mitosis and of chromosome structure. What we now have to ask ourselves is how far these secondary changes require secondary assumptions, and how far they are implied by the primary one.

Recent experiments equally with flies, infusoria and tomatoes have revealed to us the conditions of normal development. They have shown that it is characterized by adjusted rates of multiplication, adjusted not merely between nucleus and cytoplasm, but between different self-propagating constituents of the cytoplasm. These adjustments can be broken down under experimental conditions, with results which have been accurately described. In polymitotic maize, as we saw, the cytoplasm runs away from the nucleus. In polymitotic millet the nucleus runs away from the cytoplasm. In *Paramecium*, in CO_2-sensitive *Drosophila*, and in rogue tomatoes, the whole system at a high temperature can be made to run away from a particular plasmagene. By rapid growth the 250 Kappa particles of one cell can be run down to one (from which they can recover) and then finally to zero (from which they cannot recover), so that the cell lineage has mutated. And in most virus diseases of course the virus runs away from the rest of the system or *vice versa* ; only occasionally is equilibrium reached. Evidently therefore the cell contains self-propagating elements with different limits to their speeds of reproduction. These limits are exposed in a protozoan or a plant when the temperature is raised. They are equally exposed in a bird or a mammal when a plasmagene arises which lifts the speed of reproduction of the cell to a new level. Such a change will introduce a reproductive race, a *competitive propagation*. It will slowly or swiftly sort out the self-propagating elements according to their capacities, and in consequence will slowly or swiftly alter the character of the cell-lineage (Preer, 1948 ; Lewis, 1948 ; L'Héritier, 1948).

In cancer I am supposing a cytoplasmic change which favours a high growth rate. In these circumstances both the nucleus and certain cytoplasmic constituents might well be unable to stand the pace. The chromosomes might become sticky and fail to divide in time, and thus give rise either to polyploid or to hypoploid nuclei. All these indeed have been described by Koller (1947*b*) and by La Cour (1944). Certain self-propagating proteins important in differentiation and adapted to low rates of propagation of differentiated tissues might well be lost. Their loss would appear in the loss of characteristic enzyme systems, that is in secondary regressive mutation and dedifferentiation—such as the amelanosis of a melanoma or the loss of the stilboestrol reaction by prostate cells. Such changes are characteristic of cancer.

Thus, if the mutant plasmagene itself can stand the pace, its positive mutation will inevitably lead to a series of negative mutations, and the restoration of an embryonic growth rate to a cell lineage will prove to be incompatible with the maintenance of many of its specifically adult self-propagating elements.

Nor even need we expect the cancer-determining plasmagene system itself to be unaffected. Violent multiplication will be accompanied by selection favouring plasmagene combinations which determine even higher growth rates,

although changing in the means of attaining them. We must therefore expect modifications to arise in tumours as they do in viruses which are propagating themselves under optimum conditions. But these modifications will be more elaborate, since the behaviour of the cell is determined not by one but by many mutually adjusted self-propagating components.

The conditions of this selection will be determined by the genetic character of the host or victim. Just as some strains of *Paramecium* but not others are able to get rid of the Kappa plasmagene by more rapid multiplication, so some individual mammals should be able to prevent or avoid the establishment of cancer mutant plasmagenes which others cannot prevent or avoid. With some genotypes, as well as in some environments, the tumour plasmagene will not be able to keep up in the race it has started, and the tumour will come to a standstill after limited growth. Differences in susceptibility to cancer which are governed by differences of both heredity and environment bear out this expectation.

It is thus in the conditions and effects of the particle-race that we have the answer to the most dangerous objection to the plasmagene theory of the origin of cancer, namely, that it is a self-evident proposition and a meaningless truism. Where mutation depends on growth rate we have a basis for prediction and a subject for experiment.

SUMMARY.

Tumour development is a contradiction in the nature of the individual, and we can understand it only in terms of cells and particles. How we can do so has been made clear in the last five years by our increased knowledge in three directions :

1. The induction of cancer by chemical agents is now seen to be a genetic mutation, although outside the nucleus and inherently outside the germ-line.

2. Between the hereditary plasmagenes and the naturally infectious viruses an intermediate class, the proviruses, is now seen to lie.

3. These three classes of particle are conditional and interchangeable. Cancer-producing particles fall into all three.

The origin of cancer can therefore be ascribed to mutations in cytoplasmic determinants, indifferently infectious or non-infectious, which make themselves visible by causing the resumption of growth.

Further, the study of plasmagene and virus inheritance in relation to differentiation reveals a competitive propagation of cytoplasmic particles. This explains both the genetic control and the secondary development of cancer with its potential dedifferentiation and metastasis.

The discovery of, not the cause, but the system of causation of cancer tells us nothing immediately about the cure that we do not already know. But it enables us to take the cancer problem out of the lumber room of biology (for cancer has not hitherto been mentioned in the text-books) and use it as a prop and buttress for the whole subject.

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" INFECTIVE " TRANSFORMATIONS OF CELLS.

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I. ALTHOUGH biologists have for long been uneasily aware of the existence of a problem of cell heredity, its systematic analysis is the work of very recent years. Cell heredity deals with the origin and maintenance of *inherited* character differences between cells; more particularly, between cells that set up division lineages by mitotic fission. Its problems present themselves in their most acute, if not most easily workable, form in metazoan development.

The outcome of cellular differentiation in (say) mammalian development is the formation of a limited number of histologically definable cell genera, each one further subdivided into a variety of cellular genetic species. The genus " fibroblast," as yet far from completely analysed, may be subdivided into cells which manufacture bone, cartilage, white connective-tissue fibres, and so on. The genus " epidermis," of which we have made a particular study, includes the epidermal epithelium of the superficial skin, of the sole of the foot, the tongue, the claws or nails, the vagina and the cornea. Cells of each of these epidermal species display a distinctive combination of structural or physiological properties. The epidermis of the sole of the foot, for example, has a characteristically high rate of cell division. One is at first tempted to believe that this is an immediate consequence of the wear and tear and constant irritation that sole epithelium submits to. But this has proved not to be the case. Although plausible " phenocopies " of sole epithelium (in the form of corns and callosities) can be made by irritating the thin and relatively quiescent skin of the body surface, the difference between the division rates of sole and body epidermis is in fact " intrinsic " and inheritable. We have, for example, transplanted sole epidermis to positions in the body where it is protected by neighbouring hair and secure from mechanical irritation; but even so, its characteristic growth rate has been maintained for at least two years, and thick pads of now functionless cuticle continue to form over it and may periodically be removed. Claw epithelium tells the same story—more clearly, since the difference between claw and body epidermis is anatomically crude and obvious. We have recently begun a study

of corneal epithelium, as something of a test case. Corneal epithelium is very transparent, and its mode of cell-packing and cuticle formation is characteristic. These properties might well be the outcome of the peculiar situation in which it lives—lying taut over the unvascularized corneal dermis, relatively low in temperature, and nourished under conditions of low O_2 tension by the aqueous humour, and perhaps to some extent through its outer (cuticular) surface as well. If the difference between corneal and body epidermis were merely a difference of "nurture," then the heterotopic transplantation of the corneal epidermis to (say) the chest wall should bring about its reversion to the cell type characteristic of body skin. This does not happen. By grafting small corneal shavings to large granulating areas on the chests of rabbits, the corneal epidermis can be made to expand its substance and area fifty-fold by cell division and outward spread; but it still retains its characteristic cell packing, mode of cuticle formation and exquisite transparency, so that fine regenerating dermal blood-vessels are to be seen through it as clearly as through a window. Corneal grafts have proved to form a rather unstable epithelium, which never seems to achieve the firm union with the underlying corium characteristic of body skin. For this reason we have not yet been able to "cultivate" corneal epithelium as a heterotopic graft long enough to be quite sure that it represents a distinct epidermal species. We can only say that it has given no hint of reversion after five weeks of cultivation.

In general, the technique of tissue culture confirms the results of heterotopic transplantation; but it is far less critical in its discrimination between cells of different species, because cultivation *in vitro* has a tendency to make different cells look rather alike. Cultivation *in vitro* is not known to bring about any inherited change in differentiated cells, unless we take into account the fact that prolonged and rapid growth may perhaps cause individual cells to lose specific properties in the "growth rate dilution process" recently reported on by Preer (1946). The "antigenic simplification" (Gorer, 1938, 1942) and anaplastic tendencies of long established and rapidly growing transplanted tumours may conceivably represent a phenomenon of similar origin.

It will be clear from the foregoing examples that the techniques we have so far used to discriminate between the several true-breeding species of epidermal cells are, in their use of heterotopic transplantation, formally identical with those used in classical experimental embryology to distinguish between cell systems undergoing "self" and "dependent" differentiation. The epidermal species illustrate the nature of cell heredity with particular clarity because, unlike the cell systems the embryologist is accustomed to work with, they represent *final terms* of the sequence of embryonic differentiation. The nature of the comparatively small differences between these end-products of differentiation may prove easier to analyse than the radical dichotomies of early embryonic life.

II. The mere contemplation of conservative cell heredity—of a faithfully true-breeding cell lineage—can obviously tell us nothing about the mechanism of cellular inheritance. It is a truism to say that Mendelian genetics is a theory about the *differences* between individuals. Mendelian genetics (from which we exclude comparative cytogenetics) has nothing to say about any character common to all the members of an interbreeding assembly; there is a genetical theory about the ability to taste phenyl thio-urea, but not about the ability to taste salt. (It is most unfortunate that the phylum- and class-specific characters

of animals belong to the group on which Mendelian theory is, for technical reasons, obliged to remain silent; from this, no doubt, arises the lay misconception that Mendelism only "accounts for" relative trivialities.) In short, Mendelian theory is made possible by the phenomenon of segregation. The study of cell heredity is likewise, in effect, the study of cellular transformation—that is, of the origin of inherited differences between the phenotypes of cells.

From our point of view, the most important of the several different types of cell transformation is that which, in a rough preliminary classification, has been called *infective* (Medawar, 1947). To this category belong the transformations of cells by endogenous or exogenous viruses or virus-like bodies. Infective transformations have two distinctive features. The first is that the maintenance of the transformed state they bring about depends upon the continued presence in the cell of the transforming agent or a faithful copy of it. The second, a corollary of the first, is that infective transformations can be serially propagated —a cell once transformed can in its turn transform another.

The special importance of infective transformations is that they provide clear direct evidence of the *particulate* nature of cell heredity, and so, indirectly, of the fact that the differences between cell species are combinatorial in nature and not smoothly blended. The authors' work on the inheritance of colour differences in the cells of guinea-pigs' skin is relevant here. In spotted guinea-pigs (as, apparently, in spotted pigs and Friesian cattle) the pigmentation of the black skin areas can be seen during life to encroach upon the white. The spread is even in density and smoothly progressive, but it normally leaves untouched the hairs of the white area that are being encroached upon, so that the transitional zone of black skin that was formerly white is distinguished by bearing white hairs on a black skin background. Our interpretation of the phenomenon (Billingham and Medawar, 1948) is, in outline, as follows: The origin and seat of pigmentary function in black or other coloured skins is the *pigmentary dendritic cell*, a branching cell which lives in the lower reaches of the epidermis and which in some unexplained manner "feeds"—or, as Masson (1948) puts it, "injects" —melanin granules into the Malpighian layer cells on which its branches end. Pigmentary dendritic cells form a facultatively syncytial system, since the branches of neighbouring cells are sometimes confluent, and a branch from one dendritic cell, instead of ending on a Malpighian cell, may sometimes apply itself to the cell body of another.

In the white areas a dendritic cell system is present which is similar in every respect save one: the cells lack both melanin granules and the enzyme apparatus required for making it, and no form of merely physical stimulus will cause them to acquire pigmentary function.

We have shown, not yet with final precision, that the phenomenon of pigment encroachment or spread at the pigmentation boundaries of spotted guinea-pigs is due to the transformation of white (non-pigmentary) dendritic cells into melanin-forming cells by contact with their pigmentary neighbours. The transformation, once achieved, is permanent in the transformed cell and its division lineage; and a white cell transformed to pigmentary function can in its turn transform its neighbours. The process of pigment spread is thus contagiously infective; and as a crude first approximation we have suggested that the infective agent is a "plasmagene" (Darlington, 1944) type of enzyme or enzyme-complex with (in this context) a virus-like behaviour. According to our interpretation, there-

fore, the inherited difference between pigmentary and non-pigmentary dendritic cells proves to satisfy the following very simple equation :

pigmentary dendritic cell = non-pigmentary dendritic cell + pigment-forming system.

III. We have now to ask whether the pigment-spread phenomenon we have analysed is a mere curiosity of nature, or whether it exemplifies a principle of general importance.

The experimental system we have been using is a very unusual one indeed, for the following reasons : Most cells liberate their secretory products externally or into the general circulation. Dendritic cells pass *their* secretory product, melanin, into the cells around them—" cytocrine " activity, as Masson calls it. Their neighbours are normally the Malpighian cells of the epidermis ; but, as Masson has pointed out, dendritic cells may also inject melanin granules into cells of quite different origin—into the cells of gut epithelium for example, if a tumour metastasis happens to bring them within reach of its branches. In all such cases the melanin is acquired passively or at second hand, and behaves as an inert end-product. Pigment spread becomes possible only when pigmentary dendritic cells are given access to non-pigmentary dendritic cells, and these are so little different from their pigmentary neighbours that they are " competent," as embryologists say, to support the continued formation of the pigment-producing enzyme complex presumably introduced into them. Since skin homografts do not survive, so that black skin from one individual cannot be made without any special techniques to initiate pigment spread in the white skin of another, the phenomenon can only be studied in spotted animals. But not in all laboratory animals—only in those, like the guinea-pig, and unlike the rabbit or mouse, in which dendritic cells are present in the superficial epidermis. (Pigment spread does not occur in spotted rabbits or mice.) In short, an infective transformation of the type we are considering is made possible only by the coexistence in one individual of a semi-syncytial system of cytocrine cells of two species, differing from one another in a single, specific, and at the same time phenotypically conspicuous way. On the face of it, nothing could be more " specialized " or more trivially parochial in its significance.

Further reflection shows that such an interpretation would be short-sighted. It may well be that the cytoplasmic enzymes which endow the various cell species with their characteristic " phenotypic " properties are particulate self-reproducing entities ; but this cannot be *demonstrated* unless such an entity is removed from one cell and transplanted into and caused to multiply within some other cell competent to give it quarter. But the whole mechanism of development can be regarded as a conspiracy to prevent just this sort of thing's happening. The casual leakage of enzymes from one cell into another would make nonsense of embryological development ; no organ could exist under these conditions, with its great variety of cells of different types in intimate physical contact. An infective transformation can only be secured by taking advantage of peculiar cell properties, like the cytocrine properties of dendritic cells and their transiently syncytial lay-out ; of peculiar animals, like spotted guinea-pigs ; or of technical tricks, like grafting claw epithelium to the skin of the chest, or—to choose an example from the illuminating pioneer work of Sonneborn (1943*a*, *b*)—by delaying the conjugation process of *Paramecium* so that the conjugant pairs may

exchange some part of their cytoplasm as well as their gametic nuclei. It is not the plasmagene that is the odd anomalous curiosity, but the type of experimental system which, in defiance of any sense of the fitness of things, allows a plasmagene to display infective properties and so reveal itself to be an instrument of cellular heredity.

IV. The relevance of these ideas of cellular heredity to cancer etiology has been dealt with by other speakers, so that only special aspects of the problem need be considered here.

The centre of gravity of modern speculation about tumours has altogether shifted from the idea of the malignant cell as one which proliferates faster than its neighbours, towards the simpler and more general fact that it has acquired an inherited character difference from its normal homologues. Their rapid rate of growth and their invasiveness may be the most important clinical facts about tumours, but they are not necessarily the most important biological facts. Suppose, now, that we accept one variant or another of the view, that malignant cells differ from their normal homologues by the multiplication within them of an exogenous or proprietary virus or mutant plasmagene. If that view is correct, the infective propagation of tumours should be the rule rather than the rare exception. (For if we disregard the intervention of virus-like particles in the initiation of mammary tumours in mice and in the propagation of the Brown-Pearce carcinoma (Kidd, 1946), the list of tumours or tumour-like growths initiated by viruses seems to be exhausted by the Shope papilloma and a variety of chicken sarcomas.) But what has been said above about the inherent difficulty of demonstrating infective transformations in normal cells applies without any qualification to those which happen to be malignant. The limiting factor must be supposed to be, not the actual rarity of tumours rendered malignant by the propagation within them of specific virus-like particles, but our technical facilities for extracting them from malignant cells and introducing them into normal cells of a type in which they are competent to flourish ; in other words, not the material existence of such particles, but their reluctance to display infective behaviour. Luckily, infection techniques are not the only ones at our disposal ; Kidd's (1946, 1948) subtle serological analysis of the Brown-Pearce carcinoma has the great theoretical merit of overcoming their almost crippling defects, though it has certain limitations of its own.

The interpretation we have put upon the phenomenon of pigment spread in guinea-pigs' skin was said earlier to have been incompletely demonstrated for this very reason ; although we have the strongest circumstantial and anatomically factual evidence for supposing that the transformation we have been studying is infective in character, we have not yet been able to produce a cell-free extract of pigmentary dendritic cells which will convert white dendritic cells to pigmentary function. The difficulties of extraction are formidable enough ; we may be dealing with a small battery of oxidases, and the existence of at least two, a tyrosinase and a dopa-oxidase, is likely. The enzyme complex may be associated with structural matter in the cells, possibly with melanin granules too large to enter anything but a cell of phagocytic habit. Then there arises the problem of how to administer the cell extract, even supposing that a cell not normally phagocytic will admit foreign matter of high molecular weight. Should the cell be quiescent or dividing ? Will not the trauma consequent upon any form of application cause the cells to round off and become specially resistant to pene-

tration ? And so on. The pigmentary dendritic cell can infect its white neighbours, because, as a cytocrine cell, it is specially adapted to perform just that sort of function. To reproduce its action artificially cannot be technically easy ; we may be obliged in the end to resort to the crude solution offered by micro-injection methods.

In spite of the rarity of overt cases of infective propagation of tumours, it may nevertheless occur under circumstances in which, being clinically of trivial significance, it has been overlooked. If the so-called melanotic carcinoma of skin is in reality a tumour of dendritic cells, then it is at least conceivable that some melanomas spread infectively by the transformation of their non-malignant neighbours. Such spread would be so slow as to be wholly outweighed in clinical importance by infiltrative growth and metastasis, but clinicians may know of certain peculiarities of melanotic growths which such a concept would help to interpret. It would be a mistake here, of course, to suppose that the melanotic character of such growths was itself of anything but secondary importance : some melanomas are recognizable histologically which are barely melanotic. All epidermal dendritic cells of human beings, barring albinos, can be provoked into manufacturing melanin ; and an irreversible malignant or pre-malignant change might be initiated in normal dendritic cells by the cytocrine activities of their malignant neighbours before their rapid growth, and rapid elaboration of melanin, gave outward evidence of the fact.

Since this is a privileged occasion, speculation may be allowed to become wilder still. From time to time there have appeared in the literature suggestions that even epidermal tumours may initiate a slow infective spread, i.e. that whatever the nature of the primary carcinogenic stimulus, an epidermal cell may be infectively transformed at second hand by a malignant neighbour. The ordinary literature of tumour transplantation is unhelpful on this point, since stock epidermal carcinomas are normally propagated by subcutaneous (or some other form of heterotopic) transplantation from mouse to mouse. A description of the behaviour of skin tumours *orthotopically* grafted (i.e. grafted into a skin position) seems somewhat overdue, and the authors are beginning such an investigation now. The mere examination of an orthotopic graft of tumorous epidermal cells could hardly be informative ; it would be necessary to show by judicious transplantation experiments that claw or tongue epidermal cells *as such* acquired malignant properties by contact with malignant epidermal cells of a different species. It is very highly unlikely that anything of the sort would happen ; but cancer research has not yet the authority to refute such a possibility out of hand, and the current revival of interest in the infective propagation of tumours justifies its trial even without high hopes of its success.

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CHEMICALLY INDUCED MUTATION.

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THE discovery of the carcinogenic hydrocarbons arose from clinical observations on the prevalence of certain types of cancer associated with certain occupations (Potts, 1783; Cook, Kennaway, Hieger and Mayneord, 1932). Strong (1945) later showed that one of these, 20-methylcholanthrene, was also capable of inducing germinal mutations in mice, and later I confirmed this effect, using 1:2:5:6-dibenzanthracene as the carcinogen and mutagen (Carr, 1947). This, I think, raises the awkward problem that mutations may also be an industrial hazard, and I would like to lay especial emphasis on this point, as it may not receive the attention it deserves. My reasons for considering that the risk is a real one, and of importance, are perhaps best discussed in relation to my own experiments, as I am naturally most familiar with this material.

I was injecting dibenzanthracene into some dominant (lethal) yellow mice of mainly inbred type, and intercrossing for up to four generations, in order to study the types of cancers induced in such mice. As some of Strong's work had already been published, the possibility of obtaining mutations in the course of the experiment was considered, but discarded as unlikely, reasoning by analogy with X-rays. The numbers of mice involved were small, the method inefficient by reason of the loss of 25 per cent of all offspring—and therefore of mutants—in mating yellow to yellow, and many colour mutants would also be lost as they might appear on albino mice and not be detected, or be invisible on a yellow background. Therefore, in order to economize in space, I further reduced the possibility of detecting mutants by permitting some interbreeding among litters. I was thus rather startled to find that the experiment yielded not only the induced tumours that I desired, but also a number of undoubted mutations—brown, chinchilla, pink-eye, recessive spotting, hydrocephalus, and some whose genetic nature was not established—brain hernia and absence of a uterine horn. This indicated a mutation rate of one in a very few hundred for visible genes, and makes a very surprising comparison with X-rays and Drosophila, where the detection of any effect of the rays depended at first on the elaboration of " trick methods " by Muller; previous workers who had tried to use direct detection of visibles after radiation failed for reasons that are now obvious to anyone with genetical training. If the same high ratio of visible to lethal mutations had held in this work as in X-rayed Drosophila, the experiment should have been almost wrecked by sterility effects, yet these were not noticeable during the work.

Fortunately I already had some clues to the solution of this difficulty, as I had also been associated—in a very minor way—with the work on the production of mutations by the mustard compounds now used for cancer therapy (Auerbach, Robson and Carr, 1947). I do not intend to talk about these, as Dr. Auerbach

herself is here. But from this work we were already aware that there were some differences between the effects of X-rays and the mustards, and by assuming that the hydrocarbons were reacting with even less energy than the mustards, it might seem not altogether unreasonable that the carcinogens would induce mutations in rather readily-mutable visible genes rather than disrupt them into lethals or break chromosomes to give translocations and inversions.

I next attempted to determine how lasting the effect of an exposure to a mutagenic hydrocarbon would be in mice, and also to transfer the work, if possible, to the more easily handled Drosophila for quick answers to some questions. Unfortunately circumstances beyond my control interrupted this work, and it has not yet been resumed. As far as it went, the results were as follows: In mice that were essentially CBA in make-up (4th to 6th generations of a back-cross) which had been injected with dibenzanthracene six months previously, I was able to test 62 chromosome sets for visibles and found no recessives, but one variant that might have been a dominant—an F_1 mouse devoid of gonads or accessories. Even this one variant raises a suspicion that the dibenzanthracene had done permanent damage to the reproductive system, as the rather standard type of mouse used should not throw new and undescribed mouse variants.

My attempts with Drosophila were started before the results of Demerec (1947) were published, and I had the very decisive negative results of Auerbach (1939) to consider. It therefore seemed to me not impossible that the mutant effect might be due, not to the hydrocarbon, but to a metabolic product of it, and the hydroxy-derivatives described seemed rather more likely to penetrate to the gene after feeding than would the pure hydrocarbon. Professor Cook generously provided me with three of these compounds, which I mixed with food and raised some Fo-4 flies on it. The resulting males were tested by the C1B method, and gave mutations of 1·1 per cent to 2 per cent as compared with a spontaneous mutation rate of 0·4 per cent. The tests were on 400–800 F_2 males in each group. These results, though consistent, are not significant statistically unless all three treatments are combined into one group. Of some interest was the incidental finding of two visible mutants found in the casual inspection of C1B cultures, which would be an extraordinary occurrence by mere chance. The Drosophila work, too, indicated that the mutants were appearing in sperm after removal from the mutagen. The substances used were :

1:2:5:6-dibenzanthracene (3:4?) diol,
4′hydroxy-1:2-benzanthracene,
9:10-diethyl-1:2-benzanthracene-(3:4) diol,

of which 2 mg. were mixed with 5 ml. of food.

I would like to indicate here that my use of phenolic derivatives is perhaps rather comparable to Demerec's use of aerosols, as the carcinogenic hydrocarbons are rather easily oxidized to quinones in air, and such quinones would be readily reduced to the phenolic derivatives *in vivo*. This suggestion that the phenolic compounds are the active materials at once brings to mind the results of Levan and Tjoi (1948), who found that most phenols more complex than phenol itself inflicted considerable damage on plant chromosomes.

Now if these data that I have described are combined with the much more extensive material of Strong, the suggestion is that exposure to the carcinogenic hydrocarbons induces in mammals a rather gentle chemical upset of the gene,

which tends to mutate only those loci that require a relatively small energy change to give a new stable mutation. This would also imply that these compounds will tend especially to mutate genes that have already mutated spontaneously. Thus, although not really specific for any given gene, their effects may be normally limited to only a small fraction of the genes on the chromosome. But I think that there is a risk with chemicals not encountered with radiation, and this is that a given chemical can react with all, or at any rate a major part of the genes of one type in the animal exposed, as this is merely a repetition of a possible chemical reaction. Then they might also react with the same genes in another exposed individual equally well, and thus the offspring of two people exposed to carcinogenic mutagens could immediately show an induced recessive. This gives a new problem in human genetics—inbreeding with an occupation. And I have suggested that mutant sperm may be produced long after exposure to the mutagen ceases. I don't know whether any data on this could be accumulated—all I can recall in the literature is the so-called " industrial melanism " of insects described by Harrison (1920), but I think we should be aware of the risk. My results do not prove that the risk is there, but I think that they do suggest a possibility. Strong's experiments gave many dominant mutations, and these are even more serious, as they directly affect the next generation. From the mouse data, many of the mutants in humans might merely be to induced blonds, which are not regarded as completely undesirable by some, but hydrocephalus is always a human tragedy.

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INDICATIONS OF THE HERITABLE NATURE OF NON-SUSCEPTIBILITY TO ROUS SARCOMA IN FOWLS.

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UNDER a scheme sponsored and developed over a period of years by the British Empire Cancer Campaign, the birds of the inbred Brown Leghorn flock maintained by one of us (A. W. G.) at the Institute of Animal Genetics, Edinburgh, were made available to research workers using avian tumours as well as those engaged in other branches of cancer work. This allowed of the use of animals of known antecedents, together with the advantages of a more uniform genetic constitution, and it was intended to relate the information obtained about cancer growth with the pedigree data, with a view to improving our knowledge of susceptibility to cancer in order to breed fowls of known susceptibility for the investigation of special aspects of cancer research.

This scheme has made available, among other things, data on the susceptibility of fowls to the Rous No. 1 sarcoma, from the experiments by C. R. Amies and J. G. Carr, involving some 3000 birds. In addition, as will be described later, it was early found to be worth while starting a deliberate attempt to breed resistant fowls to assist in a study of this condition. This programme has involved over 1000 birds in addition to those previously mentioned.

Constitution of the Edinburgh Brown Leghorn Flock.

For almost twenty years the flock has been maintained semi-intensively under standard conditions of husbandry in a brick building designed for the purpose. The policy of keeping the birds on a standard diet was also enforced until war conditions made it impossible, and difficulty in obtaining feeding stuffs of a consistent quality is still being encountered.

No fresh blood has been introduced into the flock since 1931, and the stock has not been in contact with other birds since that time. In 1932 interest in the inheritance of egg production led to the subsequent division of the flock into a number of separate inbred lines, each selected for some special trait bearing upon production. They are still being maintained as separate entities up to the present time, and it is their differing response to tumour inoculations that provides the material for the present discussion.

Susceptibility to spontaneous tumours.

Cancer is an extremely common spontaneous disease of the domestic fowl in all parts of the world. Exact figures comparable with those for other species are rather difficult to obtain, however, for three main reasons : Firstly, since egg production falls rather rapidly with age, birds are seldom kept beyond the second year of production, and it is estimated that the average age to which birds are retained would not exceed 20 per cent of their potential reproductive life. When

it is considered that the same proportion of the reproductive life of a human female would bring her to 21 years of age, and that of a mouse to 4½ months, this emphasizes the very restricted nature of the sample usually studied.

Secondly, most of the data on spontaneous tumours comes from reports from poultry pathology laboratories, and is given as a percentage of all cases of deaths sent in for examination. This causes a serious under-estimate for several reasons. For example, many of the deaths are due to *S. pullorum* and coccidial infections which strike the immature chick long before cancer age; further, pathological investigations are usually only requested when a sudden rise in mortality suggests an epizootic is beginning, while isolated deaths, which would include those due to cancer, are not always submitted for investigation post mortem.

Thirdly, there is little agreement among poultry pathologists upon the classification of cancer. Thus some include fowl paralysis (neurolymphomatosis) and others exclude leukaemia. The data available are thus no guide to "cancer incidence" in fowls, in the sense that this can be defined for mice and man, and are, for the reasons suggested above, usually a gross underestimate. Although many such figures are available from various parts of the world it will perhaps be sufficient to compare the tumour incidence in the Edinburgh flock, where all birds dying are examined *post mortem*, with data given by Goss (1940), where a similar analysis has been made with flocks totalling 24,000 birds. Of the birds autopsied 19·5 per cent contained tumours and represented 6 per cent of the total flock numbers. Out of 675 female birds dying since 1932 in the Edinburgh flock at ages ranging from 1 to 9 years, only 50, or 7·4 per cent of the total adult mortality, gave evidence of neoplastic or allied conditions. The replacement figure to maintain flock size require the introduction of approximately 200 pullets annually, and some indication of distribution of age groups may be gained from the constitution of the flock at the beginning of this year (1948).

TABLE I.

Age :—	9 yrs.	8 yrs.	7 yrs.	6 yrs.	5 yrs.	4 yrs.	3 yrs.	2 yrs.	1 yr.	Totals.
Female population in 1948	5	9	11	13	11	81	70	169	222	591
1932–1948 :										
Neoplasms	2	..	2	4	3	5	6	7	8	37
Leukaemias	..	..	..	..	1	1	4	4	3	13
Annual incidence	..	..	..	0·9%	..	..	..	0·4%	0·3%	1·6%

Calculated on this population basis (Table I) annual losses from neoplastic and allied conditions are extremely low, being 0·3 per cent in pullets, 0·4 per cent in two-year-old birds, and 0·9 per cent for the remainder of the age groups. The Edinburgh flock in general is thus a "low cancer" line of considerable interest. At present it is sufficient to indicate that complications due to spontaneous cancer can be neglected in considering the results which follow.

A preliminary study of susceptibility.

The first analysis was made upon a series of 429 birds inoculated with cell-free Rous No. 1 virus over a period of about 2 years. These data were obtained from the results of experiments of various types (e.g. titrations of virus, antibody neutralizations, action of enzymes), and the number of inoculated sites in each individual varied accordingly, as did the amount of active virus involved. All

the data, however, referred to 6–9-week-old chicks, all inoculations were made intramuscularly, and all birds were known to have received active virus. The susceptibility of each bird was graded according to the following scheme : Negative result (0), regression of tumours (R), tumour response to 1 to 4, for increasing size and malignancy of the induced sarcoma, giving six classes in all. The largest at death or after 28 days was used as an indicator of the susceptibility of the bird. The grading was done by inspection. Weighing the tumour is unlikely to be more accurate because of the difficulty of clean dissection, and the neglect of such factors as the size and condition of the host, the invasiveness of the tumour, and the presence of large blood-clots and mucoid fluids within its substance.

In addition to differences in susceptibility of the host other possible causes of variation were considered, such as sex, season, site of inoculation, number of inoculations, dose of virus, and presence of other factors, such as antibodies injected with the virus. From an early stage, however, it became clear that these would be of only minor significance. No consistent sex difference in susceptibility was noted during the work, and, as was later seen in the analysis, sex does not seem to affect the susceptibility to any detectable extent. Though seasonal variations are important in some kinds of cancer, e.g. teratoma testis (Michaelowsky, 1928 ; Falin and Gromzewa, 1939 ; Bagg, 1936) ; transplantable sarcoma (Peacock, 1935) ; chemical induction (Murphy and Sturm, 1941), an analysis of the susceptibility of these young chicks to the Rous No. 1 sarcoma showed no seasonal trend (Carr, 1942). That the site of inoculation is of little importance in influencing tumour growth was shown by Gye and Purdy (1931) ; it was claimed by them that the resistance depends upon the dose of virus inoculation. No support for this claim could be found by Carr (1942, 1943*a* and *b*), and the results described below indicate that any such variation is inconsiderable.

That the other factors mentioned, viz. number of inoculations and presence of other materials, were probably of slight importance was concluded from several experiments in which only one of these factors varied in an otherwise identically treated group. The results clearly indicated that individual variations in the host susceptiblity would override these effects. A large number of the experiments found in the literature illustrate this point, which is seen to be confirmed in the analyses that follow.

The birds comprising the first group of tested animals were from most of the Institute lines. " All the year round " hatching had been necessary to maintain an even supply of experimental chicks, but not many matings were represented by more than a few tested chicks. In fact, the 429 birds were descended from 33 sires and 147 dams, and many matings had but one tested offspring. The detailed analysis is therefore omitted, but Table II shows the distribution of responses for

TABLE II.

Group.	Degree of response.						Total.
	4.	3.	2.	1.	R.	0.	
Breeding	99	120	57	46	11	11	344
Non-moult	4	3	5	6	14	7	39
Large egg	4	8	5	6	1	1	25
Intensity	4	4	3	4	1	2	18
Small egg	0	0	2	0	1	0	3
Total	111	135	72	62	28	21	429

the various Institute lines; these were all selected for physiological characters associated with egg production.

Dividing each line into two clear classes, negatives and non-negatives, the difference between the breeding line (with the largest number) and non-moult line was examined. The fourfold contingency table—

			Total.
Breeding . . .	333 .	11 .	344
Non-moult . . .	32 .	7 .	39
Totals . . .	365 .	18 .	383

gave a value of chi-square of 13·9 (using Yates' correction, which always makes the value smaller than without the correction).

This large value (for 1 in 100 chance = 6·63) leaves no doubt that the class Non-moult is strongly associated with no tumour. A similar test on the most conspicuous of the other negative tumour groups, Intensity, gave a value of chi-square 1·23, which is of no significance, being the variation that would occur in 1 out of 4 random samples. It was therefore unnecessary to test the rest of the groups. Since the regressions (R) are equal to the negatives in the Breeding line, but more frequent than the negatives in the Non-moult line, the frequency of regressions in the Non-moult line is therefore even more significantly disproportionate than the number of negatives, so that this line has an obviously high resistance to the production of fatal cancer by the Rous No. 1 virus.

The evidence of differences among the lines led to the examination of backcrosses to the susceptible and resistant lines, and a comparison of their responses with those of the pure parent lines and their first crosses is shown in Table III. Each of the classes represented there includes the progeny of two or more pens of birds; all the resistant (N) birds were from the Non-moult line, and all the susceptible, except one Intensity male, from the Breeding group.

TABLE III.—*Percentage of Progeny giving Responses in Grades 2 to 4.*

Mating.	Population.	% "Susceptible."
1. Susceptible × Susceptible . . .	485 .	70
2. Susceptible × Cross	71 .	69
3. Susceptible × "N"	87 .	47
4. "N" × Cross	102 .	30
5. "N" × "N"	187 .	28

In this case the percentage of birds of each class yielding one of the three highest types of response is given. While this classification is wholly arbitrary, it finds some justification in the behaviour of the N-S (non-susceptible) line later extracted from the Non-moult group, in which only about 3·4 per cent give as good a response.

From the table it can be seen that while first crosses are intermediate between the two pure-line matings, the backcrosses behave remarkably similarly to the parent groups to which they are more closely associated. While this is not the situation that would be expected if a single gene difference between the lines

obtained, it must be remembered that no selection for susceptibility or resistance as such had taken place, and the possibility of a major gene with widely differing frequency in the two populations could not be excluded. Evidence from offspring of single matings was thus necessary.

Susceptibility of chicks from individual matings.

As explained before, most of the matings which gave rise to the chicks discussed so far contained only a very few offspring. At about the close of the period when the first analysis was made, the breeding programme at Edinburgh resulted in a large proportion of chicks provided for experimental inoculations being derived from a single pen of the Breeding line. These chicks were all offspring of a single sire, F651, and dams fairly closely related to him.

The annual population of the Breeding line is larger than that in any of the other groups constituting the flock, and the method of selection used is such that they are also less inbred. In Table IV the responses of the groups of progeny from 14 females mated to F651 are illustrated. It is obvious that there is considerably variation among the groups ; in the first three the responses are mainly restricted to the two highest types, and in the fourth the same tendency is only slightly less marked. In the next six progenies (G196–G866) they are distributed fairly evenly over all the classifications except the completely negative one. The last three groups tend to reverse the order exhibited by the first three, while the offspring of the remaining bird, J1607, give responses falling mostly into intermediate categories.

TABLE IV.—*Distribution of Responses of Progenies from a Single Sire Mated to Different Dams.*

Dam.	4.	3.	2.	1.	R.	0.
G720	IIIIIIIII IIIIIIII IIIIIIIII	IIIIII	I	II	II	II
G1057	IIIIIIIII		I		II	
G161	IIIIIIIII	IIII	II	III	I	
G383	IIIIIII IIIIIII	II	IIII	IIII	I	
G196	IIIII	IIIII	IIIIIIII	III	I	
G728	IIIIIII	IIIIII	IIIIII	I	IIIII	
G422	IIIIIIIII	III	IIIIIII	IIIII	IIII	
G906	IIIIIII	IIII	IIIIII	II	IIII	I
G229	IIIIII	III	IIII	III	IIII	
G866	IIIII	I	IIII	IIIIIIII	III	I
J1607	I	I	IIIII	IIIIII	I	II
G346	I	III		IIIIII	IIIII	II
G317	III		III	IIII	IIIIII	II
G248	III		II	IIIIIII IIIIII	IIIIIIIII	IIII

This tendency for the susceptibility of chicks from certain individual matings to fall into a rather narrow range has been encountered in other pens of birds, and knowledge of its existence has been made use of both in providing susceptible chicks for experimentation and avoiding the progeny of dams whose offspring were known to give a poor response. Even when only a few chicks were tested from a single mating it was noted that the susceptibilities were frequently similar rather than scattered, and so gave an indication of the pattern to be expected from the group.

The distribution of the responses in these individual groups makes it unlikely that the genetic situation is as simple as at first envisaged, and suggests additional variation of a non-genetic character; nevertheless, it raises the possibility that a line of chicks highly susceptible to the virus could be produced by selection. The desirability of this is obvious, especially when the data in Table IV are referred back to the experiments from which the susceptibility was determined; several had been ruined by the inclusion of chicks from G248 and J1607, and much waste of labour and material could have been avoided by a knowledge of the behaviour to be expected from their progeny. Unfortunately the outbreak of war and subsequent reduction of facilities prevented attempts to establish a high susceptibility line.

Non-susceptible line.

Previous to this, selection in the opposite direction, that is towards a resistant line of birds, had been begun. It had been noted that when the Non-moult chicks included in the original analysis were grouped according to sires, one of the latter, H573, stood out as producing particularly resistant progeny (Table V). In fact, when the data from his chicks were omitted the difference between the responses of chicks from the Breeding and Non-moult lines were no longer significant. It seemed probable then that the disproportionate number of regressions and negatives produced by H573 was not due to the character Non-moult as such, but to some mechanism which can be dissociated from the physiological make-up of the Non-moult type.

TABLE V.—*"Non-moult" Sires.*

Sire.	Response. 4.	3.	2.	1.	R.	0.	Total.
F725	1	1	2	0	3	1	8
G875*	1	1	1	2	1	0	6
H573*	2	0	1	0	6	4	13
H1184	0	1	1	4	4	2	12
Total	4	3	5	6	14	7	39

* Half-brothers.

These resistant individuals are of considerable theoretical and practical importance, but their comparative rarity (4·8 per cent of the original group discussed here) previously made the study of this condition difficult. It was especially disappointing that they could only be recognized *after* injection of the

active virus, so that many desirable experiments, e.g. preliminary testing for the presence of antibody, were impossible. On the other hand, to have carried out an experiment on a large number of birds in the hope that one of the rare resistant class was included would require labour and accommodation far beyond the facilities of any place engaged in fowl tumour work at this time.

The dams of the offspring of H573 analysed in Table V were all fairly close relatives of the cock himself, and the way was thus opened for breeding a line of birds which would include a large proportion of resistant animals. It was arranged to carry out a small series of tests on the progeny of H573, and almost all the birds tested gave either very small tumours, or more often, complete regressions. From the latter the non-susceptibility line was almost immediately established, and breeding from progeny showing complete lack of tumour growth resulted in offspring which showed only minor variations in the degree of resistance in subsequent years. This is illustrated in Table VI, where a comparison is made between (A) the progeny of the same dams and sire hatched in two consecutive years, and (B) the progeny of the same dams and two different (N-S) sires hatched in the same year.

TABLE VI.—*Non-Susceptible Line.*

A. Response of progeny of same dams × same sire.

		Grade of response.						Population.
		4.	3.	2.	1.	R.	0.	
1940	%	5	..	8	63	24	..	38
1941 (1)	%	..	..	1	24	69	6	70

B. Response of progeny of same dams × different non-suceptible sires.

1941 (1)	%	..	..	..	33	56	11	66
1941 (2)	%	..	..	2	44	51	3	39

Though the numbers are not large in these tests, the proportions showing the different degrees of susceptibility have been transformed into percentages for easier comparison. It may be noted that while completely negative responses are still in a minority, the actual number of birds showing any of the three highest grades of susceptibility is even less. By far the greatest proportion of responses are either regressions or the smallest type of tumour. The slight shift towards greater resistance in the first 1941 test in both halves of the Table suggests that some environmental factor leading to a greater inhibitive effect on tumour growth may have been operative then.

When the line had been thus established, a number of females, which had been tested and found to produce resistant offspring with a male of their own line, were then outcrossed to a Non-moult cock, a descendant of the group from which the Non-susceptible (N-S) line had been extracted. The distribution of the progeny of the two matings is shown in Table VII, and it is clear that the susceptibility of the cross chicks is much greater than that of the pure N-S ones. This affords additional evidence that the association of resistance with the Non-moult factor in the original stock was fortuitous, and that it was indeed selection based on tested birds that was effective in establishing the N-S line.

TABLE VII.—*Comparison of Non-Susceptible and Cross.*

Sire.	Dam.	Tumour response.						Total population.
		4.	3.	2.	1.	R.	0.	
"N-S"	"N-S"	..	..	1	8	15	3	27
"N"	"N-S"	6	10	9	27	4	1	57

It is difficult to make a comparison between the N-S line and material used by other workers for, in contrast to mice, very little attention has been paid in the past to the study of the genetics of cancer susceptibility in fowls. The pro portions of birds reported as resistant to inoculation with Rous No. 1 sarcoma virus varies from 40 per cent (Fischer, 1927) to 4–5 per cent (Gye and Purdy, 1931). These figures may be complicated, however, by different rates of spontaneous sarcoma occurring in the population, either causing "natural" or "induced" antibodies to be formed in the tested animal. This complication is probably absent at 6 weeks (the age used here) as no antibodies are present at this stage (Amies, 1937; Andrewes, 1939; Carr, 1943*b*), though they occur in younger and older chicks. Resistance also varies with the age of the bird (Duran-Reynals, 1940; Rous and Murphy, 1914; Carr, 1943*b*), so a standard of age for testing is requisite for comparison.

In the selection experiment for high and low susceptibility to Jungherr sarcoma, carried out by Cole (1941) for example, a resistant line with only 12 per cent susceptibility was obtained, but it was found impossible to raise the high incidence line much above the original 70 per cent; the cause of this may have been acquired rather than a genetic immunity, since transmission from the immune female to the chick via the egg-yolk (Andrewes, 1939; Carr, 1944) cannot be excluded, for progeny were tested at 7–18 days, a time when yolk antibody is possibly still present in the chick. Acquired immunity was ruled out as a cause of resistance of the N-S line.

Perhaps the most useful comparison which can be made is that between the present analysis of responses of the Edinburgh flock to the filtrable tumour Rous No. 1, and those of birds from the same stock to chemical tumour inducing material as demonstrated in the work of Dr. Peacock, a report on which has been published (Greenwood and Peacock, 1945). The data, which cover the behaviour of 4 lines for the 10-year period from 1935–1944, are interesting both in their points of similarity and dissimilarity to the material already discussed: They were classified only into positive and negative groups, but the Non-moult line again showed itself the most resistant to chemically induced tumours with a susceptibility of about 30 per cent, while Breeding and Large Egg gave a figure close to 40 per cent and Intensity had the highest susceptibility with 60 per cent positives. Since the early analysis of Rous data places Breeding as highest in order of susceptibility, this difference in rank order might reflect inconsistency in the relative responses of the two lines to the different types of tumour, but it appears more likely that with continued inbreeding the Intensity line has chanced to become more susceptible. Belief that this is so has led to the majority of chicks supplied to cancer workers being mainly from this line or its crosses.

A clear difference in response to two chemically induced tumours was, however, established in Peacock's material, where the Breeding line gave a greatly increased response to a second tumour as compared with the one used originally.

The most striking point of difference between the filterable and induced tumours was the distinct seasonal swing in susceptibility exhibited by the latter. This seems to indicate that though the Non-moult line was most resistant to both types of tumour, the factors operating in respect of the two cannot be entirely the same.

SUMMARY.

An investigation of the differing response to tumour inoculations of the inbred lines of Brown Leghorn fowl maintained at the Poultry Research Centre, Edinburgh, has been in progress for a number of years. Annual losses from spontaneous neoplastic and allied conditions are extremely low. When most of the inbred lines were tested it was found that one in particular showed an obviously high resistance to the production of fatal cancer by the Rous No. 1 virus.

The distribution of the responses in groups of chicks from individual matings makes an interpretation based on a simple genetic situation unlikely, and suggests considerable variation to non-genetic causes.

A preliminary test indicated the probability that a particular male mated to fairly close relatives would enable a non-susceptible line to be established. Breeding from his progeny showing complete lack of tumour growth resulted in resistant offspring, and subsequent generations gave only minor variations.

The particular inbred group from which the line non-susceptible to Rous No. 1 virus has been derived had shown itself earlier to be the most resistant to the transplantation of chemically induced tumours.

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HEREDITY IN HUMAN CANCER.

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FROM ancient times it has been observed that cancer often occurs familially : the inheritance of tumours in human beings has been studied and discussed for centuries, and it has especially been the object of extensive systematic investigations during the last decade.

About 6 years ago, a group of collaborators at the University Institute for Human Genetics in Copenhagen decided to make an attempt to investigate the genetics of various aspects of cancer. The investigation has partly been carried out in co-operation with the Danish Cancer Registry, under the direction of Dr. J. Clemmesen, the University Institute of Pathological Anatomy, and the Radium Centre in Copenhagen and numerous Danish hospitals and clinics. The work was supported by Kong Christian X's Fond, Landsforenigen til Kraeftens Bekaempelse and Anders Hasselbalchs Fond.

These investigations include genetic experiments in mice and studies in man. Twin examinations were also initiated in co-operation with the Cancer Registry. The statistical-genealogical investigations have been performed as a series of surveys each concerning a considerable number, usually several hundreds, of probands or propositi with cancer of a certain type, e.g. cancer mammae, cancer uteri, cancer oesophagi, multiple cancer or leukaemia, picked at random from the population. These investigations were made by a number of physicians, each being a specialist in the field his study concerns. The families have been studied thoroughly, and every case of cancer in the relatives has, so far as possible, been verified ; in that respect these investigations differ from earlier, more comprehensive statistical studies in the heredity of cancer. The investigations of Jacobsen (1946) on heredity in breast cancer and of Videbaek (1947) on heredity in human leukaemia and its relation to cancer have been published. Brøbeck's study on the heredity in cancer uteri is completed, but not yet published. Mogensen's study on cancer oesophagi and Feilberg's investigation on multiple cancer are under preparation for publication.

The conclusions obtained so far, as to heredity in human cancer, may be summarized as indicated in Tables I and II.

TABLE I.—*Tumour-causing Factors.*

I. *Endogenous factors.*

1. Hereditary predisposition.
   Tumours of different forms, types and sites differ in their genetical behaviour.
   (*a*) General predisposition (general cancer, or blastoma ?, tendency).
   (*b*) Tendency to localization (localization genes, organ factors, similar histological structure).

Dominance (irregular), recessivity (?).
Polymeric or multifactor inheritance.
Homologous polymeric factors.
Later and less frequently in heterozygous.
Variation in manifestation, penetrance and expressivity. Twin investigation. Age correlation.
Variation in susceptibility or refractoriness to tumour formation or tumour transplantation.
Genes with the character of virus. Cytological changes.

2. Somatic mutation (induced or spontaneous).
3. Cytoplasmic inheritance (maternal effect).
4. Internal milieu (partly environmental).

II. *Environmental factors.*

1. Internal milieu (adaption to individual, type, race or variety, and species).
   Internal carcinogenic environment.
   Hormonal imbalance, metabolic disturbances.
   Modification of internal milieu caused by: Transplantation of eggs or embryos, milk factor (maternal inheritance ?), nutrition, age, radiation (decreased resistance), carcinogenic agents (acceleration), intoxication.
2. External milieu (*exogenous factors*).
   Irritation by trauma, chemical, thermal or ray influence, parasites, bacteria and viruses.

TABLE II.—*Hereditary Tumours.*

Benign and malignant.

Neurofibromatosis, Tuberous sclerosis, Lipomata (Phakomatoses). Adiposis dolorosa. Exostoses. Enchondroma. Enostoses. Dermatofibrosis. Fibroma plantae. Fibroma molluscum. Onychogryphosis. Atheroma. Sebaceous tumours. Xanthoma. Myoma. Cheloid. Hyperkeratosis. Adenofibroma. Adenoma. Struma. Teratoma. Melanoma. Naevus. Angiomatosis retinae et cerebelli. Angioma cavernosum. Teleangiectases (Osler and Sturge-Weber). Renal cysts (multiple cysts in several organs). Hypernephroma (malignant). Hypertrophy of prostate.

Precancerous conditions, precursors to cancer: Xeroderma pigmentosum, polyposis intestini, achlorhydria (?).

Neuroblastoma retinae. Glioma. Cancer mammae, uteri, oesophagi, ventriculi, recti. Cylindroma. Sarcoma. Leukaemia. Lymphosarcoma. Reticulosarcoma.

Most of the hereditary benign tumours are inherited as monomeric, dominant or incomplete dominant characters, e.g. the phakomatoses, exostoses and some of the fibromata, atheromata, xanthomata, angiomata and cysts. The same holds good of the precancerous conditions. Some of the benign tumours, e.g. naevi and adenomata, occur occasionally familially; the genetical basis of their hereditary predisposition, however, still remains uncertain and obscure.

As regards the various forms of cancer, e.g. cancer of the breast, uterus, stomach and rectum, their occasionally familial occurrence has been known for many years. In order to elucidate the etiologic significance of hereditary factors in these tumours, proband investigation has been carried out.

Jacobsen's (1946) study on the heredity in breast cancer includes 200 probands, (197 women and 3 men) with cancer of the breast. The investigations of the probands' families comprise the following categories of relations: parents, brothers and sisters, grandparents and brothers and sisters of the parents. Videbaek (1947) has investigated pedigrees of 209 patients with leukaemia and 200 control probands, using the same methods as Jacobsen.

The propositi-method of investigation is, of course, rather complicated and leaves room for several sources of error. It must be realized that the diagnoses and the information on the whole as regards the older or more distant groups of relations are rather unreliable. Some of the grandparents, for instance, died more than 50 years ago, and at that time, diagnostic skill was more limited and hospital records and death certificates were more insufficient than to-day. Furthermore, it is difficult to procure a comparable control, material, because the probands are normally more interested in the occurrence of cancer in the family than normal control probands are; probably that is the reason why the information concerning the distant groups of relations in Jacobsen's control material is insufficient. Also it must be considered that during recent years many cancer patients, e.g. suffering from skin cancer, get cured without knowing they have had cancer, and thus escape notice by the investigator. It should also be pointed out that the calculation of the morbidity risk is a complicated method, and cannot always be relied on. Thus a critical judgment of the results obtained through these investigations is necessary.

Various forms of cancer, e.g. lip and skin cancer, are not represented in the families of the probands, but cancer of most other forms, sites and types occurs in the relatives in about the same proportion as among the relations of the control probands, and is comprised under the collective term "endogenous cancer as a whole."

According to Jacobsen (1946), a study of the case histories of the probands gives no basis for supposing that external factors play any important role in the development of breast cancer. An excess incidence of breast cancer among the female relatives of the probands and likewise a significant excess incidence of "endogenous cancer as a whole" in all the categories of relatives, both male and female, were found. This indicates hereditary predisposition as the chief factor in the development of breast cancer.

Breast cancer is dependent on hereditary factors, and the tendency to this particular form of the disease is bound up with an inherited predisposition to endogenous cancer in general. The development of the "endogenous cancers" is probably due to a general hereditary predisposition. The localization of the tumour is determined either by hereditary or external factors. In many pedigrees the general hereditary predisposition appears to be inherited as a dominant character.

In the leukaemia proband material, the familial incidence of the disease was found to be at least 8 per cent. Several types of leukaemia (acute or chronic lymphogeneous or myelogenous, monocytic or stem-cell leukaemia) may occur in the same family. The relative frequency with which they occur is the same

for the familial cases and for the disease in general. The multiple occurrence of leukaemia in an individual family is usually not confined to some particular type, which indicates that leukaemia is probably genetically a morbid entity. Among members of a family there seems to be an age correlation as regards the onset of leukaemia. According to Videbaek (1947) leukaemia, as such, is not inherited ; it is a question of inherited disposition to the disease. Simple dominance or recessivity may possibly be excluded ; it may have a genetical basis with incomplete dominance or polymeria (homologous polymeric factors).

Hereditary diseases are generally monomeric. Hereditary predisposition to disease is generally regarded as polymeric. They are graded characters dependent on many genes, which appearing alone have but little effect. The expression of the character is furthermore dependent on the interaction between the genotype and environment.

The incidence of pernicious anaemia is significantly higher among the relatives of patients with leukaemia than among the relatives of the control probands. The relation is probably due to a common hereditary predisposition. Among the relatives of the leukaemia patients there is a significantly excessive incidence of cancer as a whole, due to a high incidence of all forms of cancer. Cancer, including leukaemia, is probably a disease entity genetically dependent on a dominant gene common for all the different forms of "endogenous cancer." According to Videbaek's opinion the development of leukaemia seems to depend on various conditions, among others, on a non-specific hereditary predisposition to cancer, which is believed to be present in at least 20 per cent of the population in general, and partly on one or several genes, the activity of which plays a role for the localization of the cancer to the leukon ; leukaemia seems to constitute an entity genetically and environmental changes influence the type of the disease. There is evidence that external factors also play a role in the development of leukaemia. The probability of the occurrence of several cases of leukaemia in the same family is rather slight ; but the risk of getting cancer in near relatives of a patient with leukaemia is as high as almost 50 per cent.

Table III shows some of the data from the breast cancer and leukaemia material in relation to cancer risk. The cancer risk figures in the cases of breast cancer, are in rather good accordance with dominant inheritance if the cancer risk in the total population is assumed to be 22 per cent, as found by Videbaek (1947) and Brøbeck. It has to be considered, however, that the cancer risk figures do not differ much in dominance and recessivity, when the cancer risk in the normal population is higher than 20 per cent.

Brøbeck investigated the families of patients suffering from cancer uteri (unpublished). Among the relatives of probands with cancer of the body of the uterus the incidence of "endogenous cancer" was high, and the relatives of probands with cancer of the cervix show the same frequency as the relations of control probands. In the families of the patients with cancer of the cervix, on the other hand, a relatively increased frequency of cancer of the oesophagus was present. In this connection it is worth while to draw attention to the similarity of the histological structure of cancer of the cervix and cancer of the oesophagus. In the families of probands suffering from cancer of the oesophagus investigated by Mogensen (unpublished data), cancer of the oesophagus was often found in a close male relative, the father or a brother of the proband. In these families, both the proband and the relative with cancer of the oesophagus

TABLE III.—*Frequency of Cancer among Relatives of the Probands with Breast Cancer and Leukaemia.*

Frequency of cancer in—	Fathers and mothers per cent.	Brothers and sisters per cent.	Siblings of parents per cent.
Breast Cancer.			
Observed cancer	24	6	12
Calculated cancer risk	62	60	33
Leukaemia.			
Observed cancer	14	5	7
Calculated cancer risk	42	41	27
Expected frequency of cancer risk assuming 22 per cent cancer risk in the population :—			
By dominance	58	60	40
By recessivity	47	54	34

were as a rule suffering from chronic alcoholism ; probably it was a tendency to alcoholism and not a tendency for cancer which was inherited, the alcoholism being the cause of cancer of the oesophagus.

In mice, cancer of the breast is more frequently inherited through the mother than through the father, but this has not been demonstrated in human cancer. Hogreffe's investigations on the inheritance of leukaemia in mice demonstrate in the filial generations a segregation in early and late cases of leukaemia, dependent on genotypic factors. Probably the disease has a tendency to occur later and more seldom in heterozygous mice (and persons ?) than in individuals homozygous with regard to the predisposition for leukaemia. This observation is in accordance with the clinical investigations concerning breast cancer and leukaemia in man. In both diseases the familial incidences seem to manifest themselves earlier than is usually the case. The differences could, however, not be significantly proved, owing to the insufficiency of the material, and the differences may be due to the fact that the old propositi generally have less detailed and exact information about their relatives in earlier generations than the young propositi have. Still the accordance of the experimental results and the clinical observations makes the significance of the latter probable.

Our investigations into the heredity of various aspects of cancer, have shown that hereditary disposition is an important tumour-causing factor. It was found that it is the chief factor in the development of breast cancer, and many other tumours are more or less dependent on hereditary factors. The general " cancer-tendency " (likely not a general blastoma tendency) occurs with considerable frequency in the population (it is probably higher than 20 per cent), and is possibly inherited as a character showing incomplete dominance. The possibility of multifactor inheritance cannot, however, be excluded. Our investigations have shown that the tendency of tumour-localization has also a hereditary basis. The development of leukaemia and cancer is probably due to a common hereditary predisposition. Previous and recent twin-investigations in unselected

materials suggest that hereditary factors must play a part in determining both the character and the site of the tumours, but that peristatic factors in a great measure contribute to determining its manifestation.

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INHERITANCE OF XERODERMA AND ITS CHROMOSOME MECHANISM.

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XERODERMA pigmentosum (hence referred to as X.P.), is marked by roughening, dryness, pigmentation and ulceration of the skin. The condition is attributed to hypersensitivity of the skin, particularly that of the basal cell layer, to sunlight. The disease was first described adequately by Kaposi in 1874 (Hebra and Kaposi, 1874). The biochemical basis of this metabolic disturbance is yet to be determined; a discussion of such an aspect of X.P. is outside the scope of the present paper.

The usual onset of X.P. occurs in the first year of life, often within a few months after birth. First, erythema develops on the skin, on those regions which are exposed to light, followed by freckling and frequently by telangiectasis. From these regions epithelioma or basal-cell carcinoma develops. The malignant change may take place not only in the skin, but also in the cornea and conjunctiva

The genetical and chromosomal basis of X.P.

The hereditary basis of X.P. was first investigated by Siemens and Kohn (1925). They analysed 76 families, and Cockayne (1933) brought this more up-to-date with a further 43 family histories. In both groups of data the incidence of consanguineous marriages was very high: 24 out of 76 and 16 out of 43 respectively, were first-cousin marriages. By analysing the family histories, Cockayne came to the conclusion that X.P. depends on a recessive autosomal gene.

The true genetical basis of X.P. was discovered by Prof. J. B. S. Haldane (1936) in his search for incomplete sex-linkage in human pedigrees. According to his analysis, the gene X.P. is one of those which is borne in that particular region of the sex chromosome, common to X and Y. It may not be superfluous to describe the cytological mechanism which underlies incomplete sex-linkage. The genetical basis of incomplete sex-linkage is clear, and is already described

by Aida (1921, 1930) in Cyprinodont fishes. More cases were reported in the genera Lebistes, Aplocheilus and Platypoecilus by Winge (1923, 1934). The cytological demonstrations of the mechanism responsible for incomplete sex-linkage was provided by Koller and Darlington (1934). They found that during the first meiotic division of the male rat, one or two chiasmata are formed between the X and Y chromosomes, and that the shape of the sex bivalent is determined by the position of chiasmata in relation to the centromere and differential segment. The latter contains the sex differentiating genes.

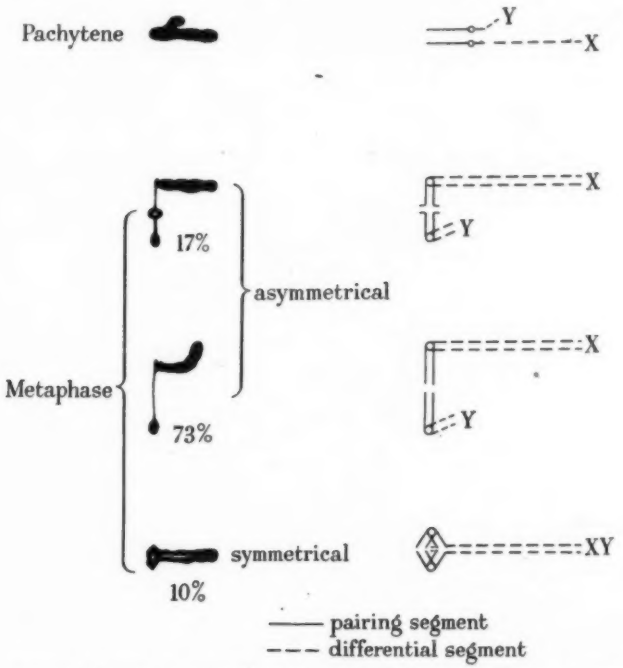

FIG. 1.—Diagram showing the behaviour of X and Y during meiosis in man. The pairing segment of the two chromosomes is indicated by a solid line, and the differential segment by a broken line. The structure of the bivalent is determined by the position of the chiasmata; the frequencies of the various bivalents are shown by the number of percentages beside the figures.

Similar chromosome behaviour was observed in man. The fact that the heteromorphic pair present in the chromosome set of the human male, forms a very easily observable unequal or asymmetrical bivalent during meiosis, is well known from the works of Painter (1923), Evans and Swezy (1929). The symmetrical configuration of the sex bivalent was identified by Koller (1936) (Fig. 1). There is no doubt that crossing-over in the distal regions of the pairing segment leads to the formation of the unequal or asymmetrical type, while crossing-over in the region which lies between centromere and differential segment, is responsible for the symmetrical bivalent.

The behaviour of XY clearly indicates that genes in the homologous or pairing segment of the sex chromosomes will exhibit crossing-over with sex. Because the frequency of crossing-over is a function of distance, genes, therefore, which lie near to the differential segment would behave as completely sex-linked, while those further away would behave in very nearly the same manner as autosomal genes. When a man inherits such incompletely sex-linked gene from his mother, it will be in his X chromosome, but occasionally it may cross-over to the Y; consequently the man will transmit the gene to most of his daughters and to a few of his sons. Similarly, if he inherits it from his father, he will transmit it to most of his sons and to a few of his daughters.

Human genetics is greatly indebted to Prof. J. B. S. Haldane (1936) for clarifying the true genetical basis of X.P. He analysed 82 family histories collected from various sources, and by using ingenious statistical methods drew the conclusion that the incomplete sex-linkage of the gene X.P. with sex is of a high degree of probability. From recombination data Haldane determined the locus of X.P. gene in the pairing segment, and placed it at 14 units from the differential segment between the gene *achromatopsia* and *Oguchi's* disease. R. A. Fisher (1936) has made further statistical analysis, and shifted the locus to 18·26 crossing-over unit. He made the suggestion that one of the 82 families on which analysis was based should be excluded on internal evidence, which would further loosen linkage, and in this case the true position of X.P. gene would be 25·19 (Fig. 2).

Family histories of X.P.

Since 1944 three family histories have been collected in which one or more members were afflicted with X.P. The data obtained from these cases has given further information on the hereditary basis of this morbid condition, and has shown the significance of the pathological symptoms, including the degree of manifestation and the time of onset of the disease. The presentation of the family histories is arranged in their order of importance.

(1) *The F— family.*—In February, 1945, Rita F— was aged 10 when she attended the Royal Cancer Hospital for treatment of a skin lesion on the calf of the left leg. The following information was obtained from her parents: At the age of one year the whole skin of the left side of her face came up in red blisters after being exposed to the sun. She was treated with calomel lotion. At the age of three she developed a wart-like lesion on the left cheek. The condition was diagnosed as X.P. It was treated by "caustic pencil," and six months later, when it recurred, by radium. In 1943 another lesion developed on the right cheek, which was similarly treated by radium. The patient's skin of the exposed parts (face, hands, legs) shows some scarring. Both the pathological skin conditions and the histological analysis of the lesions indicated X.P. The lesion on the calf has undergone malignant transformation and was diagnosed as squamous-cell carcinoma.

The patient has one brother, two years younger, who is normal. The parents are unrelated; father is of a dark complexion, mother has some slight freckling on the cheek, which is of the common type. Search through family histories of near relatives and sibs did not reveal any similar conditions. The parents are well aware of the herditary nature of the condition, and anxious not to have more children.

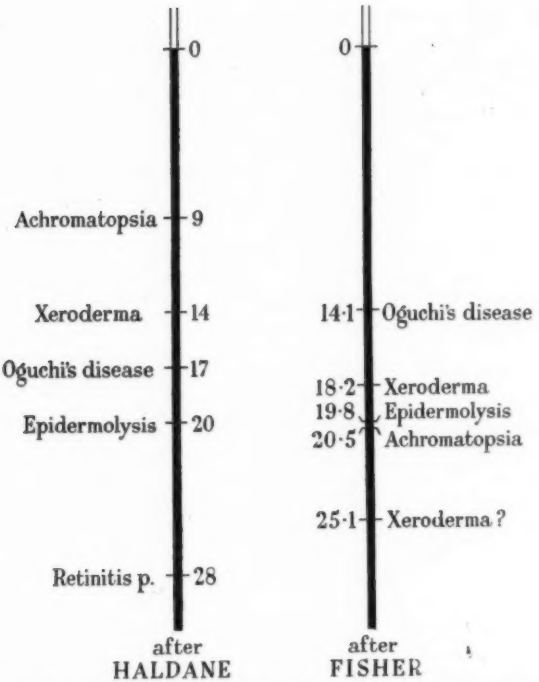

FIG. 2.—The genetical map of the pairing segment of the sex chromosomes according to Haldane and Fisher. The position of the following genes is indicated: *achromatopsia*, *xeroderma*, Oguchi's disease, *epidermolysis* and *retinitis*.

(2) *The T— family*.—In November, 1946, Gillian T—, aged 8, attended the Royal Cancer Hospital (Fig. 3, 4). Her history of childhood is similar to that of Rita F—. Excessive freckling appeared on the face and forearm at the age of three. The lesion on the left side of the upper lip was diagnosed as squamous-cell carcinoma, originated from X.P., and was treated by X-rays. Her brother, two years older, is well, no sign of similar skin condition. The parents are first cousins, and both normal; the maternal and paternal grandfathers are brothers (Fig. 5). In order to explain the manifestation of X.P. in the female sex, it must be assumed that crossing-over has taken place between the X and Y chromosomes in the father and grandfather. For that reason the pedigree of the T— family is of particular importance.

(3) *The H— family*.—In April, 1945, Isabel H—, aged 64, attended the Joint Consultation Clinic at the Royal Free Hospital, complaining of freckling of the skin, and ulceration. Although freckling had started in early childhood, it only became troublesome when the patient was 30 years of age. At that time some of the freckles developed into small ulcers, which healed slowly. She found out, by experience, that strong sunlight accentuates the lesions, and since the age of 40 she has taken care not to expose herself to direct sunlight. At the age of 61 the patient received radium treatment for several lesions distributed in five

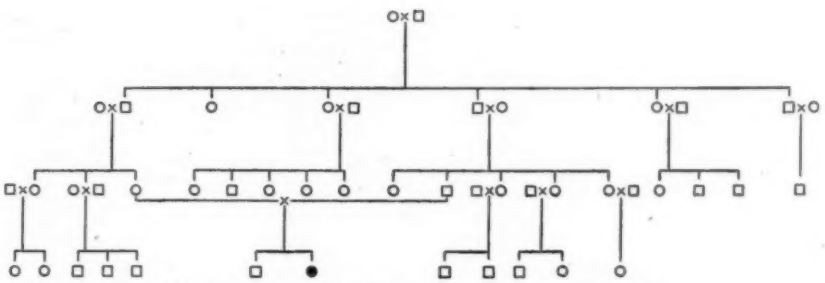

FIG. 5.—The pedigree of the T— family. Xeroderma pigmentosum.

● female affected.
□ male normal.
○ female normal.

different areas on the face. At the time of consultation the skin of exposed parts of the hands, forearms and face showed scarring, freckling and hyperkeratosis (Fig. 6). White scarred areas were present on the face, indicating the regions of previous ulcers. X-ray treatment was given to two lesions in the Royal Cancer Hospital. The skin conditions and histological diagnosis indicated X.P., except that the symptoms were manifested in a much milder form than usually is the case. The other sister, Dorothy, has a very similar condition (Fig. 7). Owing to the co-operation of the patient, the pedigree of family and relatives was traced through six generations (Fig. 8).

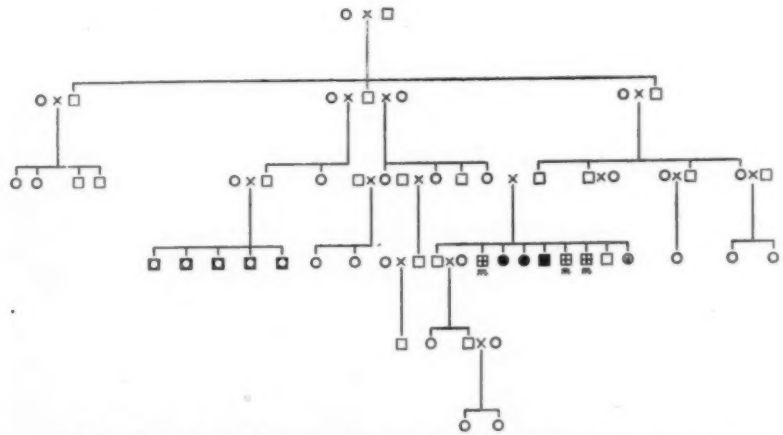

FIG. 8.—The pedigree of the H— family. Xeroderma pigmentosum (mild form).

○ female normal.
□ male normal.
● female affected.
■ male affected.
◻ sex unknown.
⊞ miscarriage.
⊙ lived one day.

The parents were first cousins; neither of them had freckling or other abnormal skin conditions. The maternal and paternal grandfathers were brothers. The eldest brother, Frank, his children and grandchildren are all normal. Isabel, the patient concerned, was the second child, but before her birth one miscarriage took place. Her sister, Dorothy, is one year younger; she is afflicted with the disease in the same mild manner as Isabel. Dorothy shows freckling on the face and forearm, which is somewhat less severe than that of her elder sister.

A younger brother, Thomas, was born after Isabel. He developed freckling on the face at the age of 20. Slight ulceration on his face and forehead were treated two years later by radio-therapy. Thomas died at the age of 23 by an accident. The next brother, Sidney, was born ten years after Thomas; between them two miscarriages occurred. The high number of miscarriages and the death of the sixth child, a girl, on the day following birth, may, not unlikely, find its explanation in the consanguineous marriage. Sidney died at the age of 25; up to his death he appeared to be normal.

There are two unusual features in the history of the H— family which require explanation. One is the late onset and mild manifestation of the disease, which is known to appear within a year or two after birth. The other fact is the relatively high number of cross-overs amongst the affected members. Because the gene of X.P. is transmitted through the maternal and paternal grandfathers, both affected sisters must be the result of crossing-over.

The probability of obtaining such a segregation as was found in the H— family was calculated for both loci (14, Haldane, and 25·19, Fisher) of the X.P. gene (Table I).

TABLE I.

Number of types of families.	Male.		Female.	
	Normal $1-p$	Affected $1-p$	Normal $2-p$	Affected p
A . 1	0	3	2	0
B . 6	1	2	1	1
C . 3	2	1	0	2
$p = 0{\cdot}25$	5	3	7	1
$p = 0{\cdot}125$	9	7	15	1

The chance of obtaining the various classes is as follows:

$$\text{Class A} = 1 \times (1/16)^5 \times 5^0 \times 3^3 \times 7^2 \times 1^0 = 1323 \times (1/16)^5$$
$$\text{Class B} = 6 \times (1/16)^5 \times 5^1 \times 3^2 \times 7^1 \times 1^1 = 1890 \times (1/16)^5$$
$$\text{Class C} = 3 \times (1/16)^5 \times 5^2 \times 3^1 \times 7^0 \times 1^2 = 225 \times (1/16)^5$$

The probability of obtaining a segregation of Class C is 0·065 when $p = 0{\cdot}25$; and is 0·0142 when $p = 0{\cdot}125$. From the statistical analysis it appears that the segregation of X.P. in the H— family does not conform closely to expectations; the number of cross-overs is higher than could be expected.

In order to explain the exceptional pathological features and the unexpected segregation of X.P. in the sexes in this particular family, the following possibilities may be considered: (*a*) This condition may be due to an independent autosomal gene, or (*b*) the gene X.P. may have been transferred to a more distal region

FIG. 3 and 4.—Gillian T—: xeroderma with squamous-cell carcinoma of the lip.
FIG. 6.—Isobel H—: xeroderma with squamous-cell carcinoma on nose (infra-red photography).
FIG. 7.—Dorothy H—: xeroderma with slight freckling and hyperkeratosis.

of the pairing segment. The increased distance of the gene from the differential segment would loosen linkage with sex, and its altered position may also lead to a lower degree of malignancy.

The data at our disposal from this one family, however, is too meagre to draw definite conclusions, whether we are dealing here with a distinct autosomal gene or with position effect. By presenting the case with its unusual features we wish to emphasize that it is of great importance to collect exact information when the medical profession deals with a hereditary disease. In this way only can we hope to bring together reliable data on which geneticists could construe a better genetics of man.

SUMMARY.

Xeroderma pigmentosum is marked by roughening, dryness, pigmentation and ulceration of the skin, which consequently leads to malignant transformation. According to Haldane it depends on an incompletely sex-linked gene.

Three family histories are presented in which one or more members of the family are affected with xeroderma. In two families the usual pathological symptoms were observed, while in the third the condition was manifested in a mild form. Furthermore, it was found that the segregation of xeroderma in this family does not conform closely to expectations.

This behaviour suggests that the condition in this particular family may be due to an independent autosomal gene, or to position effect, i.e. the gene is transferred to a more distal region of the pairing segment of sex chromosome.

I am greatly indebted to Prof. D. W. Smithers (The Royal Cancer Hospital, London) and to Dr. R. T. Brain (Children's Hospital, Great Ormond Street, London) for the cases, and to Prof. K. Mather (University, Birmingham) for his help in the statistical analysis.

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TWIN STUDIES AND OTHER GENETICAL INVESTIGATIONS IN THE DANISH CANCER REGISTRY.

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From the Danish Cancer Registry under The National Anti-Cancer League, Copenhagen.

Given at the Symposium on the Genetics of Cancer, London, June 24 and 25, 1948.

THE Danish Cancer Registry, founded in 1942 by the National Anti-Cancer League, and working in close collaboration with the National Health Service, is an organization with the primary intention to register all cancer cases, for research purposes. This is done through a voluntary system ; all hospitals notify their cancer cases to the registry, and these notifications are supplemented with death certificates for all persons who are known to have suffered from malignant diseases, including leukaemias, myelomata and brain tumours.

From Clemmesen's studies on occupational mortality from cancer in Denmark it was clear, already before the start of the Registry, that a complete investigation of the occurrence of cancer demanded at least an estimation of the role played by heredity in the origin of various cancers. It was thought to be especially valuable to carry out such an investigation alongside the mapping out of cancer incidence with regard to age, occupation, and other variables, and to compare the hereditary tendency shown by cancers of various sites in the same population and at the same time.

So collaboration was established with Professor Kemp's Institute for Human Genetics supported by mutual grants. Dr. Jacobsen (surgeon) investigated the heredity of breast cancer, and Dr. Videbaek (physician) studied leukaemia, in collaboration with the Institute for Pathological Anatomy. Dr. Brøbech (radiologist) compared the heredity of cervical and body cancers of the uterus, and Dr. Feilberg (surgeon) is analysing the genetical basis of multiple cancers. The financial support is given by grants from King Christian X Fund.

We believe that the results so far obtained have been more encouraging from an oncological than from a genetical point of view. While the methods employed have made it possible to show a practically demonstrable hereditary tendency to breast cancer, followed by a tendency to cancers of all sites, and while leukaemia and cancers of all sites show similar features, the mode of inheritance is difficult to work out, because of the less reliable information obtainable from relatives more remote than sisters, brothers or parents of the patients.

An efficient examination of the mode of inheritance could no doubt be carried out by means of a registration system for cancer patients covering a few generations. So far our registration system has not been made complete, but in order to exploit fully the opportunities at hand we have examined—as a link in the system of genetical investigations—the occurrence of cancer in twins among the cancer cases notified from Danish hospitals in the first six years of the registry, 1942–1947, realising that an unselected sample of such cases would be of some interest.

HEREDITY IN BREAST CANCER AND LEUKAEMIA.

Jacobsen, in his monograph *Heredity in Breast Cancer*, examined the occurrence of cancer in the familes of 200 breast cancer cases from the files of the Cancer Registry, and compared them with 200 control families (Table I).

TABLE I.—*Jacobsen's Breast Cancer and Control Propositae. Observed and Computed Number of Cancer Cases among Relatives.*

(200 *breast cancer cases.*)

Relatives.	Breast cancer propositae. Number of cases of cancer.				Control propositae. Number of cases of cancer.			
	All sites.		Breast cancer.		All sites.		Breast cancer	
	Obs.	Comp.	Obs.	Comp.	Obs.	Comp.	Obs.	Comp.
Fathers	40	23			19	22		
Brothers	16	8			4	10		
Maternal grandfathers	17	22			6	20		
Paternal grandfathers	13	20			5	19		
Maternal uncles	24	23			3	21		
Paternal uncles	15	25			1	31		
Male relatives	125	121			38	123		
Mothers	55	29	21	7	19	27	2	4
Sisters	30	16	13	5	8	12	2	2
Maternal grandmothers	23	33	4	7	4	29		4
Paternal grandmothers	11	26	4	6	3	27	2	4
Maternal aunts	51	43	17	11	6	34		5
Paternal aunts	30	30	12	7	3	26	2	4
Female relatives	200	177	71	43	43	155	8	23

Nearly all cancer cases among the relatives were checked by means of case-records from hospitals or death certificates. This applies, without exception to the nearer relatives, and both to test and control material. As there was some doubt as to the absolute validity of this control method, we computed the number of cancer cases which would be expected, based on the morbidity figures from the Cancer Registry and on the official mortality statistics. The data of Table I show that the value of Jacobsen's control material is rather doubtful with regard to the groups of more remote relatives. For the groups of closer relatives, as parents and sibs, however, it is fully justified to draw conclusions with regard both to breast cancer and to cancer of all sites, both of which show a definite excess for relatives of breast cancer cases.

Videbaek, working on cases with leukaemia (Table II), made a careful collection of control material. It is clear from our analysis that he succeeded to some extent only; but the data show that there is still a significant excess of cancer among fathers and sisters of leukaemia patients.

TABLE II.—*Videbaek's Leukaemia and Control Propositi. Observed and Computed Number of Cancer Deaths among Relatives.*

(209 *leukaemia cases.*)

Relatives.	Leukaemia propositi.			Control propositi.		
	Obs.	Comp. I.	Comp. II.	Obs.	Comp.	Obs./comp. per cent.
Fathers	31	20		18	19	95
Brothers	18	15		9	14	66
Maternal grandfathers	21	21		17	21	79
Paternal grandfathers	22	22	14	13	26	50
Maternal uncles	31	28	17	20	29	68
Paternal uncles	26	30	19	18	30	59
Sons		1		1	1	
Male relatives	149	137		96	140	68
Mothers	28	24		21	22	95
Sisters	32	18		15	17	87
Maternal grandmothers	23	27		27	29	92
Paternal grandmothers	22	28	17	16	23	69
Maternal aunts	33	40	24	22	36	61
Paternal aunts	31	40	25	20	32	62
Daughters	1	1		1	1	
Female relatives	170	178		122	160	76
All relatives	319	315		218	300	73

These investigations into the heredity of breast cancer and leukaemia illustrate that even under very favourable conditions which prevail in a small country, and with easy access to families interested in such investigations, it is very difficult indeed to obtain a satisfactory control material. Nevertheless, the data so far obtained show that there is a definite hereditary tendency to breast cancer, combined with a tendency to cancers of all sites, and a corresponding phenomenon was found with regard to leukaemia and cancers of all sites.

It has been reported by various authors that in families with a hereditary predisposition for cancer, the disease will appear at an earlier age in the younger generations. Thus Videbaek found an " anticipation " in material gathered from the literature, but it can be demonstrated on his own material as well as on

Jacobsen's that a higher age of the propositus was followed by a loss in the number of known grandparents, which indicates reduced reliability of even family information from older propositi. Moreover, the younger generation will show a higher number of diagnosed cancers and leukaemias as a consequence of the development of diagnostic facilities. The difference in age at onset of the disease in different generations may, therefore, be due to the method of observation itself, as is shown by the fact that leukaemia and cancer cases observed at about the same time but in different generations will exhibit age differences.

Jacobsen observed that propositae among whose relatives he found cases of cancer were younger than propositae with families without cancer, and he assumed that this was due to hereditary factors. Another explanation, however, seems to be more obvious, viz. that the old propositae are unable to give detailed information about their relatives in earlier generations because these relatives most often died years ago. This assumption is supported by the fact that most of the old propositae are grouped by Jacobsen amongst those with " no hereditary predisposition."

We find it very doubtful whether anticipation and an earlier occurrence of hereditarily predisposed cancer cases can be demonstrated by means of materials collected as those published by Jacobsen and Videbaek. Material collected in a registry does not seem to have the same shortcomings, but a further and presumably final study of such phenomena would, however, demand a cancer registration through a longer period, and at present this has not been achieved anywhere. Yet, our material seems to offer a good opportunity for studies on cancer in twins (and already from the start of the genetical team-work such an investigation had been prepared).

HEREDITY OF CANCER IN TWINS.

It seems particularly advantageous that we have at our disposal an unselected material which is rather unusual for twin studies of this kind. It comprised originally all hospital cancer cases from the Danish population of 4 million through six years, or an annual number between 5000 and 6000 cases, but this figure must be reduced by about 20 per cent in which the question about twin birth had been left unanswered, giving a total very near to 30,000 cases (29,458).

The system of notification involves a rather heavy toll of omissions, yet it has functioned satisfactorily. The special questions asked twins with cancer—besides such items as name, address, occupation and the like—were confined to previous diseases of a more serious character, including diagnosis and the date, and place of treatment. In order to identify whether the twins were monozygotic or dizygotic, questions were asked about the similarity and about mistakes made by parents or others, and similarly about type and colour of hair, colour of eyes, stature, height and weight. On the whole it may be said that we demanded almost full congruity before accepting a pair as monozygotic. The final distribution of dizygotic twins on groups of same and opposite sex, and the proportion between monozygotic and dizygotic pairs seem to show that our measures have been justified. It should be stressed that we have not been in a position to contact the patients or their twins personally. We have had to rely on information from the hospital doctors, and, on the whole, our confidence in them has been justified. A number of cases in which both twins were affected,

however, had to be discarded as a result of special inquiries to hospitals about the cases. Out of a total of about 30,000 hospital cases notified for cancer we found 315 pairs of twins, or 1·07 per cent. The official percentage of twin births in Denmark for the years 1926–30 is 1·64, and considering the increased mortality among twin babies we think this a satisfactory correspondence.

Another check on the result was obtained through computation of the number of deaths expected from all causes among twins for whom notifications were satisfactory. Among monozygotic twins we expected 20·8 deaths against 16 observed. Among dizygotics we expected 36·3 and found 26. These differences are not statistically significant, and they may be partially due to the less efficient information about those pairs of which one partner has died. In Denmark, as elsewhere, the girl-boy ratio at birth is 0·97 with a male excess, which in the course of a few years changes to a slight female preponderance. However, we find among both monozygotic pairs and dizygotic pairs of opposite sex a female preponderance of 1·48 to 1, and 1·64 among the same-sexed dizygotic pairs, making 1·53 for the total. The explanation of this considerable female excess in our material no doubt must be ascribed to the higher cancer incidence among females than among males in Denmark, combined with the earlier age in which female cancers arise. And, if we calculate the percentage of opposite-sexed twin pairs in our material, a figure which should be uninfluenced by the difference in mortality among the two sexes, we find exactly the same value as in the corresponding official birth statistics—36·2 per cent.

In spite of these satisfactory results, it should be remembered that in dealing with twin-pairs, of which at least one is suffering from old-age disease like cancer, we must expect insufficient information from twins in cases where the partner died in childhood. Therefore we have excluded the twin-pairs where one partner has died before the age of 5 years. Similarly we have lost sight of a number of "second" twins through emigration and similar events, so that the number has shrunk from 336 to 185 pairs. The distribution of the final figures will be evident from Table III.

TABLE III.—*Distribution of Cancer in Twins.*

Total number twin pairs.	Number analysed.	Monozygous. Males. Total number.	Monozygous. Males. Concordant.	Monozygous. Females. Total number.	Monozygous. Females. Concordant.	Total number of concordant. Obs.	Total number of concordant. Calc.
336	185	21	2	31	5	7	5·0

Dizygous. Same sex. Males. Number.	Same sex. Males. Concord.	Same sex. Females. Number.	Same sex. Females. Concord.	Opposite sex. Males* Number.	Opposite sex. Males* Concord.	Opposite sex. Females.* Number.	Opposite sex. Females.* Concord.	Total number of concordant. Obs.	Total number of concordant. Calc.
25	1	41	2	27	1	40	3	7	9·2

* Sex of primary twin.

The observed and calculated number of twins with concordant cancer are given. The data show that there is no significant difference between the com-

puted and observed numbers. In computing the figures for expected cancers we have paid attention to the age distribution of the cancer cases, and elimination of the difference in this respect between the two groups reduces the difference to below the limits for statistical significance. There may be a tendency to a higher cancer incidence among monozygotic twin partners of cancer patients than among partners of dizygotic cancerous twins, but the increase in frequency is not statistically significant.

It might seem natural to relate the figures of seven twin-pairs with concordant cancers found in each of the two groups monozygotic and dizygotic to the total numbers of the monozygotic (52) and dizygotic (133) pairs, in which case we come to the conclusion that the cancer risk among monozygotic twin partners of cancer patients is twice as heavy as the risk among dizygotic, but such a conclusion would not be justified.

If we consider the diagnoses of cancers with regard to site of the tumour in the seven concordant cases of cancer in dizygotic twins (Table IV), we find that only one shows a tumour of the same site in both partners (Pair No. 7).

TABLE IV.—*Dizygotic Twins. Both Affected.*

Number.	Sex.	Age and condition of— Notified twin.	Other twin.
1	M.	63 C. prostatae. (hist.)	55 C. ventriculi operat. (hosp.)
2	F.	60 C. recti. (hist.)	ca. 50 Tumour mammae operat.
3	F.	52 Tumour mediastini. (hosp.)	50 C. mammae. (hist.)
4	F., M.	61 C. mammae, c. metast. ad lymphonod. (hist.)	58 C. solid. epithel. oris. (hist.)
5	F., M.	47 C. colli uteri. (hist.)	41 Carc. bronchi dx. p.m.
6	F., M.	81 C. recti inoperab. c. metast.	78 Leukosis lymphoid. Sternal marrow exam.
7	M., F.	60 C. recti. (hist.)	58 C. recti inoperabil. c. metast. (hosp.)

"Hist." signifies histological examination.
"Hosp." signifies report from the hospital treating the twin.

When the monozygotic twins are analysed (Table V) we obtain another impression: No. 1, 2, 3 and 6 pairs have cancer of similar type. The first pair is interesting in that both members have sarcomata. The second pair is a better case than would be expected from the diagnosis "incipient" cancer of the first member. The histological description of the second member does not leave

TABLE V.—*Monozygotic Twins. Both Affected.*

Number.	Sex.	Age and condition of— Notified twin.	Other twin.
1	M.	53 Tumour malign. dorsi c. metast. ad pulm. (hosp.)	52 Fibrosarcoma cutis cruris (hist.)
2	M.	43 C. labii incipiens. (hist.)	31 C. labii recidivans. (hosp.)
3	F.	65 C. mammae bilat. c. metast. ad columnam	49 C. mammae c. metast. (ad pulm. ?)
4	F.	65 C. corporis uteri. (hist.)	62 C. meatus acust. dx. et cavi tymp. c. metast. ad lymphonod. (hist.)
5	F.	75 c. coli sigmoid.	ca. 65 Gastric cancer
6	F.	68 Naevocarcinoma dorsi (hist.)	67 C. basocell. anguli oculi (hist.)
7	F.	76 C. renis sin. (urography)	69 C. ventriculi adenomatos. coll. (hist.)

"Hist." signifies histological examination.
"Hosp." signifies report from the hospital treating the twin.

any doubt of the malignant nature of the change. The third pair also shows cancer of the same organ, and even if we may question whether the tumours in pair No. 6 are homologous or not, the tendency to cancer formation in the same site seems to be more pronounced among monozygotic than among dizygotic twins.

Against the argument that these numbers are very scanty, it should be stressed that the importance of such findings depends on the size of the material from which they were selected—in this case 30,000 cancer patients. We must admit that there may be a slight selection in favour of the accessible sites. More information was obtained about twins with accessible than with inaccessible cancer. It is believed that it may be due to the fact that accessible tumours, more often than inaccessible, are treated in the radio-therapy centres, and thus become available for analysis in higher numbers.

SUMMARY.

(1) The material used for genetical investigation, having been collected during six years from a population which at the same time has been subject to registration of all cancer cases, is considered to be unselected.

(2) The data obtained indicate a tendency to a higher incidence of cancer among partners of monozygotic cancerous twins than among partners of dizygotic

twins with cancer, but the deviations from the expected values are not statistically significant if the age distribution of the material is taken into account. However, there is a clear tendency for the tumours in monozygotic pairs to affect corresponding organs in both partners, whereas this is not the case among dizygotic twins.

(3) There is good reason to believe that continued examinations along this line will be able to demonstrate an increased tendency to malignant diseases as a whole among partners of monozygotic twins with cancer, but if we are to evaluate inheritance for cancers of single site we can only hope to achieve this by cancer registration covering a longer period.

FAMILY HISTORIES OF 459 PATIENTS WITH CANCER OF THE BREAST.

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From the Royal Cancer Hospital (Free), London, S.W. 3.

Given at the Symposium on the Genetics of Cancer, London, June 24 and 25, 1948.

SINCE 1944 we have taken a detailed family history from all patients reporting with carcinoma of the breast. We have 459 family records up to the end of 1947 which are reasonably complete. So far we have not succeeded in supplementing these histories, taken at the time of first visit to hospital, by later questions to the surviving patients or by arranging visits to their homes or relations. Some attempt has been made to confirm the causes of death of relatives by letters to hospitals, doctors and the Registrar-General's department, but such confirmation has, as yet, been obtained in only a few cases.

OUR figures, therefore, contain all those errors of inadequate information and faulty recollection that one would expect from data collected in this way. There are, however, reasons for believing that they represent an under-estimate rather than an over-estimate of the cancer incidence in these families. Many people die of cancer without their relations knowing that this was the cause of death. It is in fact surprising how successful a woman can be in concealing a cancer of the breast from her nearest relatives living in the same house with her. It is not, therefore, so surprising that more distant relatives, or even close relatives living away from home, may be unaware of the nature of the illness involved Relations, even some of an older generation, who are still living at the time that the patient is first questioned may later develop malignant disease. Many patients with cancer of the breast survive for long periods following treatment, and may ultimately die from some other cause; we are, however, unable to include patients living who either have, or have had, cancer in our figures for comparison with expected incidence in the general population because no adequate morbidity statistics are available. In our series, for instance, there are the same number of sisters alive following treatment for cancer of the breast as there are sisters who died of the disease. Comparisons must be made on a basis of mor-

tality, and Professor Penrose, who is speaking about this, will include some of our figures in the comparison between observed and expected deaths from cancer of the breast and from other forms of cancer that he has made. The fact that they show an unduly high incidence of cancer of the breast and not of other cancers does seem to me to be worthy of notice.

Of the 459 families analysed, the patient questioned had to the best of her knowledge no evidence of a family history of cancer in 292, and reported family cancer in 167. Of these 167, 76 were said to have had cancer of the breast. In 54 cases a history of cancer in more than one member of the family was obtained.

The 167 patients with a known family history of cancer stated that they knew of this on their mother's side only in 88, on their fathers' side only in 34, amongst brothers or sisters in 33, and on both paternal and maternal sides in 12. This gives a maternal side history of 100 cases to a paternal of 46.

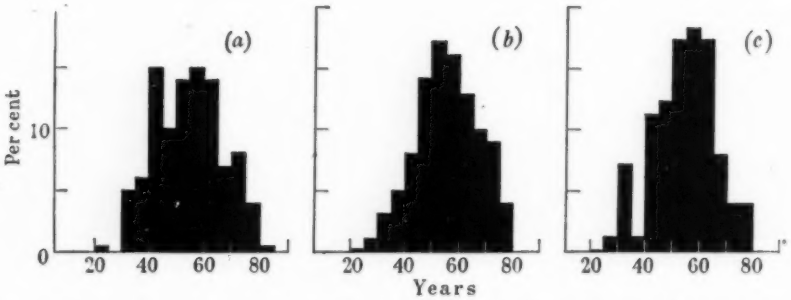

FIG. 1.—459 cases cancer of breast. Frequency of distribution at different five-year age periods. (*a*) Cases with no hereditary predisposition to cancer. (*b*) Cases with hereditary predisposition to cancer. (*c*) Cases with hereditary predisposition to cancer of breast.

Of 459 mothers, 66 were known to have died of cancer, and of these 25 had cancer of the breast. Of 459 fathers, 30 were known to have died of cancer, but none had cancer of the breast.

There were 1008 sisters, 288 of whom had died, 59 in infancy, and another 200 of causes believed to have been other than cancer. Of the 29 known to have died of cancer, 11 had cancer of the breast. There were also 11 sisters living who had received treatment for breast cancer.

There were 1059 brothers, 425 of whom had died, 75 in infancy, and another 332 of causes believed to have been other than cancer. 18 had died of cancer, but none had had cancer of the breast. One was alive following treatment for cancer.

Dividing the patients into groups according to whether they gave any family history of cancer, a history of cancer of the breast or no such family history, and distributing them according to their age at time of diagnosis we were unable to confirm Jacobsen's (1946) finding that the age of onset of the disease was lower in those with cancer in the family (Fig. 1). In our group, in fact, the average age was higher in the cancer family group, though not significantly so. The average age at diagnosis with no cancer history was 54 with a standard error of average of 0·7, with a cancer history was 55·3 with a standard error of average of 0·9,

and with a history of cancer of the breast was 54·1 with a standard error of average of 1·27.

It is to be expected that patients giving a family history of cancer at the time of diagnosis will tend to have a higher average age than the remainder

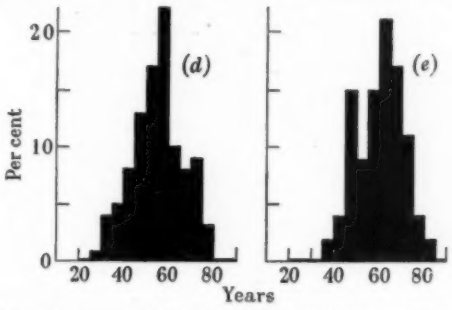

FIG. 2.—169 cases of breast cancer relative frequency at five-year age periods. (*d*) Cases in which father and or mother died of cancer of breast. (*e*) Cases in which brother and or sister died of cancer of breast.

because the brothers and sisters of the younger patients are less likely to have reached the " cancer age " than those of the older patients. Patients reporting cancer in brothers and sisters were, in fact, found to be in the older age-groups in our series as compared to those reporting cancer in fathers, mothers, uncles and aunts (Fig. 2). The average age of those reporting brother and sister cancer was 59·7 with a standard error of average of 1·54, and those reporting mother

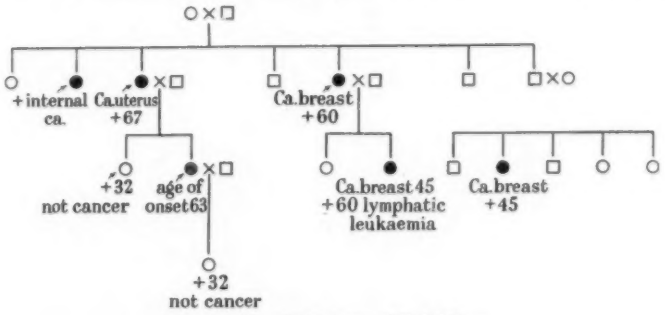

FIG. 3.—Family history of E. McC—.

and father cancer was 54·6 with a standard error of average of 1·04. This is a significant difference, whereas the average age of those reporting older generation cancer (54·6) is close to that of those reporting no cancer history (54·0).

It is of some interest to examine the family histories of a few of these patients in more detail, though care must be taken not to draw false general conclusions from individual families.

Fig. 3 shows a family where the mother of the patient died of cancer of the uterus aged 67—she had two sisters, one who died of " internal cancer," and one

of cancer of the breast, the latter aged about 60. This sister had a daughter who had her breast amputated for cancer aged 45, and who died aged 60 of lymphatic leukaemia. The youngest brother of the patient's mother had a daughter who died from breast cancer at about 45. The patient herself developed her breast tumour at 63—she had an only child, a daughter, who died at 37 after a thyroid operation for a non-malignant condition.

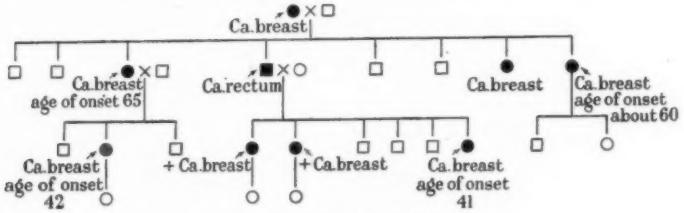

FIG. 4.—Family history of V. L—.

Fig. 4 shows a family where the maternal grandmother of the patient died of carcinoma of the breast. She had eight children, five sons and three daughters. All three daughters developed carcinoma of the breast, the age at onset being between 60 and 65. One of the sons died of carcinoma of the rectum. The oldest of these sisters was the patient's mother. The patient herself developed carcinoma of the breast at 42. One of the other sisters had no children; the other had a son and a daughter, neither of whom have developed cancer up to date. The brother with cancer of the rectum had three daughters and three

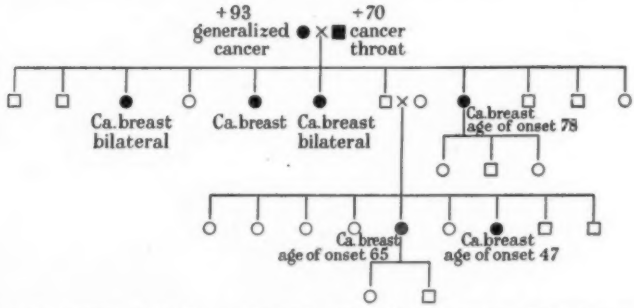

FIG. 5.—Family history of E. N—. Mother's sister died of cancer of the breast and this sister's daughter of cancer of the throat.

sons. All three daughters have had carcinoma of the breast, the age at onset being between 40–50. There are here three families, every female member of which has developed cancer of the breast, and a fourth family with one daughter in the early forties who is being kept under observation. In this particular case the age of onset of breast cancer in one generation was in the 60's, and in the next in the 40's.

Fig. 5 shows a family where both paternal grandmother and paternal grandfather died of cancer, the grandmother aged 93 of "generalized cancer"—the site

of primary was not known, but was quite possibly in the breast—and the grandfather aged 73 of cancer of the throat. There were eleven children, five sons and six daughters. Of these, none of the sons had cancer, but four of the six daughters had cancer of the breast, bilateral in two cases. The age of onset of only one of them is known, and this was 78. (This lady is also a patient of ours, and is still alive and well at 93.) One of the patient's sisters had cancer of the breast, the age of onset being 47. In addition to this, the patient's maternal aunt died of cancer of the breast, and the daughter of this aunt of cancer of the throat.

SUMMARY.

A preliminary analysis of our data suggests that there is a significantly high death-rate from cancer of the breast in the families of patients with that disease, but no higher death-rate from other forms of cancer than would be expected in the general population. We find no evidence that cancer of the breast tends to develop earlier in patients whose relations are known to have suffered from the disease.

The greater part of the work presented in this paper was done by Dr. P. Rigby-Jones and Dr. H. O. Hartley, whose valuable assistance made this contribution to the symposium possible. I am also indebted to my colleagues on the staff of the Royal Cancer Hospital whose patients were interrogated during this investigation, and particularly to Mr. R. C. B. Ledlie for his special help and interest.

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A GENETICAL STUDY OF HUMAN MAMMARY CANCER.

L S PENROSE, H. J. MACKENZIE AND M. N. KARN.

From the Galton Laboratory, University College, London.

Given at the Symposium on the Genetics of Cancer, London, June 24 and 25, 1948.

THE study of the mammary cancer forms a most advantageous starting-point for the investigation of the hereditary factors in cancers, because it is more easily recognized and more accurately diagnosed than malignant growths at most other sites. Moreover it produces, in the great majority of patients, a clearly defined range of pathological conditions, which include spheroidal-celled carcinoma, Paget's disease of the nipple, adeno-carcinoma, and the typical scirrhous condition in long-standing cases.

The history of investigations into the genetic aspect has been summarized by Jacobsen (1946). Some important records of pedigrees, showing apparent transmission from mother to daughter, were published nearly a century ago. The most remarkable was recorded by Broca (1866), in which four generations of females had the disease.

In spite of evidence brought forward by a great many observers that mammary cancer can frequently be found affecting several of the female members of a family group, doubt still remains as to whether heredity plays any significant aetiological part. This doubt arises because of the great prevalence of the disease in the general population, where it is responsible for nearly 3 per cent of all female deaths. With so common a condition, it is difficult to demonstrate convincingly that the occasional familial concentration is not merely the result of a random distribution of cases.

To obviate this uncertainty, most investigators in recent years have collected a series of cases, noted the incidence of similar disease among their relatives, and compared it with the corresponding incidence among a control group of cases free from the disease, selected so far as possible at random. In spite of elaborate precautions, the difficulties, involved in obtaining a satisfactory control group of family histories to match those of the propositae, have repeatedly proved almost insuperable. The result is that many workers (Hanhart, 1943; Passey, 1942) still consider that there is insufficient evidence to prove the reality of familial transmission of mammary cancer or of any type of human malignant disease. Stocks and Karn (1933) found no evidence that a history of cancer in relatives increased the risk of cancer to any measurable extent.

Several extensive surveys have been published, all of which give some evidence for believing that mammary cancer is unusually prevalent among the mothers and sisters of affected cases. Among the important recent investigations are those of Lane-Claypon (1926) on 508 propositae, Wainwright (1931) on 784, Martynova (1936) on 201 and Jacobsen (1946) on 200 propositae. In all these

investigations, however, some uncertainty remains as to whether the incidence of cancer cases, ascertained in the control populations, is correct. The carefully chosen control group examined by Jacobsen contained indeed far fewer cases than would be expected to occur on the hypothesis that the mortality within the group is the same as that of the general population. The result of these doubts is to reduce considerably the force of demonstrations which might otherwise seem very convincing.

The Present Investigation.

The survey now described was undertaken with two main objectives. In the first place it was hoped that a direct comparison of deaths from different types of cancer in the relatives of a series of propositae could be compared directly with the rates given in statistics of the general population. This should remove some of the uncertainties inherent in collecting a control group for comparison, and make possible fairly accurate inferences about the existence or otherwise of familial concentration of cases. Secondly, the material was compiled in such a way that, if the result should show definite evidence for the hereditary transmission of mammary cancer, indications of the type of inheritance could also be obtained. In particular, the possibilities of transmission through maternal milk or the cytoplasm were considered as possible hypotheses. Inheritance of a factor causing special predisposition of certain sites in some families but not in others was kept in mind also as a possibility.

The material, which was collected, started in each instance with a patient attending a clinic after the diagnosis of mammary cancer had been made by a competent surgeon or pathologist and, often, by both. The patients were attending either University College Hospital or the London Hospital, and they were personally interviewed. All possible hospital records and reports were searched or checked by correspondence. This procedure applied both to the propositae and to any of their relatives who were reported to have suffered from malignant disease. Unfortunately, in a number of instances, records had been destroyed by enemy action during the war. Ideally, the death certificate of every relative believed to have died of malignant disease should have been obtained, as corroborative evidence, but this we have not been able to do. The routine investigation of family histories was confined to each patient's parents, brothers and sisters, children, grandparents, uncles and aunts. These data, it is hoped, may prove to be more valuable than those previously collected in that, with careful questioning, the year of death as well as the age was obtained. This information was complete for the mothers who had died, less so for the sibs and fathers.

Altogether 521 histories of cases of mammary cancer were collected. In nine of these the diagnosis was considered to be unreliable and they were rejected. There were, also, two male cases and these were set aside ; in neither was anything of note found in the family history. The 510 remaining cases were analysed from a number of different points of view.

Analysis of the sample.

The mean age at onset of the disease in the 510 cases was 51·7 years, with a standard deviation of 11·4 years. The distribution is set out in Table I.

TABLE I.—*Age of Onset of Disease in Propositae.*

Age.	Total number.	Number with at least one affected relative.
20–	1	—
25–	5	2
30–	21	4
35–	43	11
40–	70	13
45–	101	25
50–	72	19
55–	59	14
60–	64	15
65–	38	11
70–	25	1
75–	9	1
80–	2	—
85–	—	—
All ages	510	116
Mean (years)	51·7	51·0
Standard deviation (years)	11·4	10·4

In the pedigrees of 116 instances, one or more relatives with mammary cancer were ascertained. The distribution of these 116 "familial" cases and the mean age of onset of their illness (51·0 years) was extremely similar to that of the remaining "non-familial" cases. This finding does not support Jacobsen's assertion that familial cases have an earlier onset than others. Among the group of 510 women, 408 were married but 91 of these had no children. The infertility ratio, 91 out of 408, or 22·3 per cent, significantly exceeds the value of 17·3 per cent for married women of the same mean age given by the Registrar-General (1938), and supports the view that nulliparity is an etiological factor.

The mean age of the patients when first interviewed for purposes of this survey was 55·2 years. This indicates that they had, on the average, lived three-and-a-half years since first signs of the disease had appeared. Undoubtedly the survival of the patient was a property, which determined her inclusion in the investigation, and the longer the survival, the more probably would she be selected as a proposita. On the whole, therefore, there has been a slight, unavoidable tendency to deal with cases in whom treatment has been comparatively successful. Conversely, many of them were still under radiological treatment, and others were attending hospital on account of recurrence, so that some cases may have been inadvertently selected on account of undue severity of symptoms. These considerations must be kept in mind when interpreting the data, particularly when considering the distribution of cases with respect to age of onset.

Of the 510 propositae, 255 were affected first on the left side and 253 on the right side; in two instances there was uncertainty on this point.

Familial data: mothers.—The mothers of 408 patients were ascertained to have died and, in the case of 25 of these women, death was due to mammary

cancer. Other types of malignant disease accounted for the deaths of 51 of the mothers of propositae. The date of death and the age at the time were recorded for each case. In a few instances the dates were approximate, but could hardly be more than a few years in error. The expected proportion of deaths due to mammary cancer and other types of malignancy in each year and at each age can be obtained on the basis of the general population statistics. An example of such results is shown in Table II.

TABLE II.—*Expected Proportion of Deaths Due to Cancer. Females, England and Wales*, 1925–29.

Age group.	Other types of malignancy.	Mammary cancer.
0–9	0·0023	0·0000
10–19	0·0115	0·0000
20–24	0·0143	0·0003
25–29	0·0243	0·0037
30–34	0·0543	0·0131
35–39	0·0903	0·0298
40–44	0·1409	0·0526
45–49	0·1754	0·0653
50–54	0·1938	0·0632
55–59	0·1917	0·0521
60–64	0·1807	0·0408
65–69	0·1613	0·0294
70–74	0·1384	0·0216
75–79	0·0979	0·0168
80 and over	0·0533	0·0122
All ages	0·1243	0·0239

Thus the expected number of deaths from these two causes among the mothers of the propositae could be calculated. The distribution of deaths by year and age is shown in Table III.

From this table the number of deaths due to mammary cancer if the women had been subject to the mortality in the general population was estimated at 11·17, a figure significantly less than the observed 25. For other types of malignancy the mortality among mothers of propositae showed no significant increase above expected total, i.e. 51 observed as compared with 48·76 expected.

There were 102 mothers still living at the time of investigation and, of these, six were known to be under treatment for mammary cancer while four had some other type of malignant disease. No correlation between age of onset of breast cancer in mothers and age of onset in patients could be demonstrated.

Familial data : sisters.—A similar survey of the patients' sisters revealed that, out of a total of 1255 individuals, 365 had died, though the cause and date were unknown for 58 of these. For the remaining 307 deaths the ages and dates were classified as shown in Table IV. Calculating the expected number of deaths due to mammary cancer, as with the mothers, led to a value of 6·98.

TABLE III.—*Distribution of Deaths of Mothers of Propositae by Age and Date.*

Age.	Date. '80–	'85–	'90–	'95–	'00–	'05–	'10–	'15–	'20–	'25–	'30–	'35–	'40–	'55–	All dates.	(A.)	(B.)
20–24	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	—	—
25–29	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	—	—
30–34	..	5	2	1	2	1	1	..	..	..	..	..	..	..	12	1	—
35–39	1	3	2	1	3	2	1	2	..	..	1	..	..	..	16	—	—
40–44	..	..	2	3	1	3	3	2	..	..	..	..	..	..	14	1	1
45–49	..	..	3	..	3	2	2	2	1	1	..	1	..	..	15	—	2
50–54	..	1	..	4	1	3	3	4	5	5	2	..	..	..	28	7	4
55–59	..	1	..	1	1	3	3	3	3	5	2	2	2	..	26	4	3
60–64	..	..	..	1	2	6	12	3	1	9	4	10	6	..	54	11	7
65–69	..	..	..	..	2	..	5	5	4	10	7	13	6	1	53	8	3
70–74	..	..	..	..	..	..	4	7	5	12	4	18	4	4	58	9	3
75–79	..	..	..	..	..	2	3	5	8	10	3	7	11	3	52	4	2
80–84	..	..	..	..	..	..	1	2	2	5	6	7	6	4	33	4	—
85–89	..	..	..	..	..	..	..	2	4	2	2	9	9	3	31	1	—
90–94	..	..	..	..	..	..	..	..	..	2	7	..	2	2	13	1	—
95–99	..	..	..	..	..	..	..	..	..	..	1	..	..	..	1	—	—
All ages	2	10	9	11	16	22	38	37	33	61	39	67	46	17	408	..	..
(A)	—	1	—	1	1	2	5	7	2	7	5	17	3	—	..	51	..
(B)	—	—	1	1	—	3	2	4	1	4	1	3	4	1	..	..	25

(A) Due to types of malignancy other than mammary cancer.
(B) Due to mammary cancer.

TABLE IV.—*Distribution of Deaths of Sisters of Propositae by Age and Date.*

Age.	Date. '75–	'80–	'85–	'90–	'95–	'00–	'05–	'10–	'15–	'20–	'25–	'30–	'35–	'40–	'45–	All dates.	(A.)	(B.
Infancy	17	21	14	15	8	14	10	2	1	1	..	..	..	..	..	103	—	—
10–14	..	2	1	1	1	3	..	3	..	..	..	..	..	..	..	11	—	—
15–19	..	..	..	1	..	2	1	2	2	..	..	..	..	..	..	8	—	—
20–24	..	..	..	4	2	..	1	4	2	..	1	..	..	..	..	14	1	—
25–29	..	..	1	..	..	..	..	2	2	2	3	..	..	..	..	10	—	—
30–34	..	..	..	..	1	2	..	3	1	4	1	4	4	2	..	22	2	2
35–39	..	..	..	..	..	..	..	1	..	2	2	..	1	..	1	7	—	1
40–44	..	..	..	..	..	..	1	..	3	1	2	..	1	2	1	11	2	1
45–49	..	..	..	..	..	..	..	1	1	2	1	2	3	2	..	12	2	3
50–54	..	..	..	..	..	..	..	..	..	2	7	3	2	7	3	24	3	5
55–59	..	..	..	..	..	..	..	1	..	..	..	7	4	5	1	18	2	4
60–64	..	..	..	..	..	..	..	..	..	..	..	4	5	8	7	24	5	5
65–69	..	..	..	..	..	..	..	..	..	..	..	4	3	5	8	20	1	1
70–74	..	..	..	..	..	..	..	..	..	..	..	2	5	5	3	15	1	1
75–79	..	..	..	..	..	..	..	..	..	..	..	..	..	1	5	6	—	—
80–84	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	2	—	—
All ages	17	23	16	21	12	21	13	19	12	14	17	26	28	39	29	307	..	..
(A)	..	..	..	..	..	..	..	1	1	1	..	4	7	2	3	..	19	..
(B)	..	..	..	..	..	..	..	..	..	1	1	5	5	10	1	..	..	23

(A) Due to types of malignancy other than mammary cancer.
(B) Due to mammary cancer.

Actually 23 had died of this condition. Only 19 had died of other types of malignant disease against an expected number of 25·21. Again, there is a highly significant increase in deaths from mammary cancer, even more marked than for mothers, but no significant divergence from expectation in respect of deaths from other types of malignancy.

Among the 890 sisters still living at the time of investigation, 24 were known to have been under treatment for breast cancer and seven for other types of malignant disease. Again, the incidence of these conditions among the living relatives echoes the general effect shown by analysis of causes of death.

Investigation of the age of onset in the 47 affected sisters in these families in a single entry table showed a significant correlation, + 0·57, with age of onset in propositae. It is uncertain how far this effect is real because patients, coming under observation in the younger age-groups, are not likely to have many sisters old enough to have developed the disease in late life. Moreover, the correlation of age of onset of other kinds of malignant disease in 26 sisters showed a similarly positive value of 0·79. Hence any likeness of onset age of mammary cancer in sisters is not peculiar to this type of malignancy.

Another interesting correlation concerns the sites affected in pairs of sisters and other relatives. The laterality of initial lesions showed a strong tendency to similarity in sisters and the figures are set out in Table V. For mothers, there

TABLE V.—*Laterality of Mammary Cancer in Relatives.*

Propositae.	Mothers.					Sisters.				
	Right.	Both.	Left.	Unknown.	Total.	Right.	Both.	Left.	Unknown.	Total.
Right side	7	1	7	1	16	12	—	10	3	25
Left side	2	—	10	3	15	4	1	16	1	22
Total	9	1	17	4	31	16	1	26	4	47

was a similar tendency, which by itself was not strong enough to excite attention. However, the figures for mothers and sisters together are notable enough to suggest that the tendency towards homolaterality in families is real.

Familial data : fathers and brothers.—As an additional check on the method used for estimating the proportion of deaths attributable to malignant disease, the data on fathers and brothers of the propositae were analysed in the same way that data on mothers and sisters had been. Only one case of male mammary cancer was found among these relatives but even this must be regarded as very exceptional in so few families. The observed number of male deaths from other types of cancer was not significantly different from expectation among either fathers or brothers. The results are set out for comparison with those based upon the female relatives in Table VI.

Specific transmission.

The evidence from the mothers and sisters, fathers and brothers of the propositae, as summarized in Table VII, strongly suggests that transmission of a specific factor is a major cause of mammary cancer. The hypothesis of inheritance of special organic disposition suggested by Bauer (1925) is supported by the homolateral familial findings. That theory, however, implies a significant decrease in the incidence of malignancy of other types in these families, which was not found in the present survey. Nor was there any increase in the incidence of cancer generally, which might have suggested a general hereditary predisposition to malignancy of any type.

TABLE VI.—*Incidence of Cancer Cases in Relatives of* 510 *Propositae.*

Relative.	Status.	Number of persons observed.	Mammary cancer cases.		Other malignant diseases.	
			Observed.	Expected.	Observed.	Expected.
Mother	Living	102	6	—	4	—
	Dead:					
	Cause unknown	0	0	—	0	—
	Cause known	408	25	11·17	51	48·78
	Total	510	31	—	55	—
Sister	Living	890	24	—	7	—
	Dead:					
	Cause unknown	58	0	—	0	—
	Cause known	307	23	6·97	19	25·23
	Total	1255	47	—	26	—
Father	Living	73	0	—	1	—
	Dead:					
	Cause unknown	17	0	—	0	—
	Cause known	420	0	0·10	41	52·32
	Total	510	0	—	43	—
Brother	Living	780	0	—	2	—
	Dead:					
	Cause unknown	64	0	—	0	—
	Cause known	455	1	0·05	29	28·14
	Total	1299	1	—	31	—

TABLE VII.—*Summary of Analysis of Deceased Relatives of* 510 *Propositae.*

Relative.	Total number of deaths.	Number due to mammary cancer.		Number due to other types of malignancy.	
		Observed.	Expected.	Observed.	Expected.
Mother	408	25	11·17	51	48·78
Sister	307	23	6·97	19	25·23
Father	420	0	0·03	41	52·32
Brother	455	1	0·02	29	28·14
Totals	1590	49	18·19	140	154·47
χ^2		52·1		1·2	

The familial incidence among sibs is not high enough to suggest any Mendelian explanation of the inheritance. The consanguinity test for rare recessive genes gave negative results. Three propositae had first cousin parents, and two had

parents more distantly related. These findings are in close agreement with figures found by Bell (1939) for all types of hospital patients.

In view of the work on mice by Bittner (1937), a factor derived from maternal cytoplasm, transmitted by way of milk, colostron or cytoplasm of the ovum might be a specific cause. If this were so, maternal relatives should be more frequently affected with mammary cancer than the corresponding paternal relatives. In the present series no calculation could usefully be attempted of the expected incidence in such relatives of the propositae. However, among maternal aunts there were 29 mammary against 21 other cases of malignant disease, whereas among paternal aunts only 16 cases against 24 of other types

TABLE VIII.

Malignant disease in relatives.	Mammary.			Other.		
	Aunts.	Grand-mothers.	Total.	Aunts.	Grand-mothers.	Total.
Maternal line . .	28	9	37	21	23	44
Paternal line . .	17	2	19	24	6	30

were found. These figures, given in Table VIII, are suggestive though scarcely conclusive evidence of maternal line inheritance. They derive some slight support from the investigation of maternal and paternal grandparents, where a relative excess of mammary cancer among maternal grandmothers was noted.

In these somewhat distant relatives the unreliability of paternal line information causes difficulty of interpretation. In both the parental and grandparental generations, however, sisters are more intensely affected than mothers, so far as breast cancer is concerned, and this is a fact of some genetical importance. In Mendelian genetics it would be attributed to a recessive tendency on the part of the causal gene, but it may be due to a constitutional effect associating infertility with liability to the disease in question.

A search for evidence that mammary cancer can be inherited through maternal milk gave negative results, although a more accurately planned study with this point especially in mind might show a different picture.

SUMMARY.

A series of 510 cases of mammary cancer in females was studied. Family investigations showed that the same disease occurred with significantly increased frequency in sisters and mothers of these propositae. The rate of malignancy of other types in these relatives was not increased. A specific genetical agent, responsible for the disease, is postulated, which may be inherited mainly through the maternal line.

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